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THE Committee appointed by the *Royal Society* to direct the publication of the *Philosophical Transactions* take this opportunity to acquaint the public that it fully appears, as well from the Council-books and Journals of the Society as from repeated declarations which have been made in several former *Transactions*, that the printing of them was always, from time to time, the single act of the respective Secretaries till the Forty-seventh Volume; the Society, as a Body, never interesting themselves any further in their publication than by occasionally recommending the revival of them to some of their Secretaries, when, from the particular circumstances of their affairs, the *Transactions* had happened for any length of time to be intermitted. And this seems principally to have been done with a view to satisfy the public that their usual meetings were then continued, for the improvement of knowledge and benefit of mankind: the great ends of their first institution by the Royal Charters, and which they have ever since steadily pursued.

But the Society being of late years greatly enlarged, and their communications more numerous, it was thought advisable that a Committee of their members should be appointed to reconsider the papers read before them, and select out of them such as they should judge most proper for publication in the future *Transactions*; which was accordingly done upon the 26th of March, 1752. And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them; without pretending to answer for the certainty of the facts, or propriety of the reasonings contained in the several papers so published, which must still rest on the credit or judgment of their respective authors.

It is likewise necessary on this occasion to remark, that it is an established rule of the Society, to which they will always adhere, never to give their opinion, as a Body,

upon any subject, either of Nature or Art, that comes before them. And therefore the Thanks, which are frequently proposed from the Chair, to be given to the authors of such papers as are read at their accustomed meetings, or to the persons through whose hands they received them, are to be considered in no other light than as a matter of civility, in return for the respect shown to the Society by those communications. The like also is to be said with regard to the several projects, inventions, and curiosities of various kinds, which are often exhibited to the Society; the authors whereof, or those who exhibit them, frequently take the liberty to report, and even to certify in the public newspapers, that they have met with the highest applause and approbation. And therefore it is hoped that no regard will hereafter be paid to such reports and public notices; which in some instances have been too lightly credited, to the dishonour of the Society.

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PHILOSOPHICAL TRANSACTIONS.

I. *On the Fructification of Neuropteris heterophylla*, BRONGNIART.

By ROBERT KIDSTON, F.R.S., L. and E., F.G.S.

Received November 17,—Read December 3, 1903.

[PLATE 1.]

EVIDENCE has been slowly gathering during the last few years that some carboniferous plants, which had previously been classed as Ferns, differ from true Ferns in many important points, and that perhaps a more correct definition of these fossils would be “fern-like plants.” For this group POTONIÉ has proposed the name of *Cycadofilices*.*

Until quite recently the group has depended entirely on characters derived from the anatomical structure of the stems of the plants which had been placed in it, as none of the numerous fructifications or seeds which occur in the rocks along with the stems of the *Cycadofilices*, could definitely be referred to any individual of the group.

Early in the present year, Professor OLIVER and Dr. D. H. SCOTT communicated a paper to this Society, “On *Lagenostoma Lomaxi*, the seed of *Lyginodendron*”;† and though the seeds there described were not organically united to *Lyginopteris* (*Lyginodendron*) *Oldhamia*, the structure of the glands on the cupule which surrounds the seed and the organisation of the bundle in the pedicel agree so entirely with the corresponding structures in *Lyginopteris Oldhamia*, that one can scarcely doubt the correctness of the conclusion arrived at by these botanists, that *Lagenostoma Lomaxi* is the seed of *Lyginopteris Oldhamia*. Their investigations have shown that “in the most general relations” of the organisation of *Lagenostoma Lomaxi* “the seed approaches the Gymnospermous type.” There is, therefore, in *Lyginopteris Oldhamia*, which cannot be distinguished in its vegetative organs from *Sphenopteris Höninghausi*, BRONGT.‡ a type of plant combining Cycadean as well as Filicinean characters.

It seems, therefore, impossible to doubt any longer that many of our so-called carboniferous Ferns belong to the same transitional type, and it has been shown by RENAULT§ and GRAND'EURY|| that the petioles of some *Alethopteris*, *Neuropteris* and

* POTONIÉ, ‘Lehrb. d. Pflanzenpaläontologie,’ p. 160, 1899.

† ‘Roy. Soc. Proc.,’ vol. 71, p. 477, 1903.

‡ BRONGNIART, ‘Hist. d. Végét. Foss.,’ vol. 1, p. 199, Plate 52.

§ RENAULT, “Étude du Genre *Myelopteris*,” ‘Mém. présentés par Divers Savants à l’Acad. d. Sciences de l’Institut de France,’ vol. 22, No. 10, p. 21, 1876.

|| GRAND'EURY, ‘Flore Carbon. du Départ. de la Loire et du Centre de la France,’ p. 130, 1877.

Sphenopteris have a *Myelopteris* structure, a structure which WEBER and STERZEL* have shown was possessed by the petioles of *Medullosa*, and which is further illustrated in the petioles of *Medullosa anglica*, SCOTT, whose stem exhibits the typical organisation of the *Cycadofilices*.†

The chief point which still remains to be determined is the nature and form of the pollen members of the various individuals of the *Cycadofilices*, for the presence of a seed demands the presence of an antheroid member for its pollination. Dr. SCOTT seems to favour the view that these may be found in *Calymmatotheca*, and this view may ultimately be found to be correct, but there is at present no satisfactory evidence to support it. My own opinion is that in *Calymmatotheca* we are dealing with *Sporangia*. One point is certain, that in the Lower Coal Measures, where *Lyginopteris Oldhamia* (or *Sphenopteris Hönninghausi*) occurs, *Calymmatotheca* must be very rare, as I have not met with a single specimen from that horizon, and in the Middle Coal Measures, where *Sphenopteris Hönninghausi* is also found, I can only remember of having seen two specimens, both belonging to the *Calymmatotheca asteroides* LESQX. sp.‡

Neuropteris heterophylla, BRONGNIART.

Plate 1, figs. 1-9.

The late Dr. STUR was the first botanist to express any doubt as to *Neuropteris* being a true Fern,§ and this opinion he based on the fact that no fruiting specimen of *Neuropteris* had ever been discovered, and as many other fossil Ferns showed their fructification on the fronds, had *Neuropteris* possessed an ordinary form of Fern fructification, in such a common and widely distributed genus surely some trace of it would have been discovered. Of course there was the possibility of the barren and fertile fronds being quite dissimilar in appearance, a not uncommon occurrence amongst carboniferous Ferns.

A few years earlier, RENAULT published his Memoir on the genus *Myelopteris*, in which he adopted the opinion that the affinities of *Myelopteris*, some of which were referable to the *Neuropterideæ* (*Neuropteris* and *Odontopteris*), were with the *Marattiaceæ*.|| This opinion was accepted generally as fixing the place of *Neuropteris*

* WEBER and STERZEL, "Beitr. z. Kenntnis d. *Medullosæ*," 'Bericht. d. Naturwis. Gesell. zu Chemnitz,' vol. 13, p. 43 (84), 1896.

† SCOTT, "On *Medullosa anglica*, a New Representative of the *Cycadofilices*," 'Phil. Trans.' B, vol. 191, pp. 81-126, 1899.

‡ See ZETTLER, "Observations sur quelques fougères des dépôts houillers d'Asie Mineure," 'Bull. Soc. Bot. de France,' vol. 44, p. 199, 1897; also 'Éléments de Paléobot.,' pp. 83 and 129.

§ STUR, "Morphologie und Systematik der Culm- und Carbonfarne," p. 6, 1883 ('Sitzb. der k. Akad. der Wissensch.,' I. Abh.).

|| RENAULT, 'Étude du Genre *Myelopteris*,' loc. cit., p. 21.

NEUROPTERIS HETEROPHYLLA, BRONGNIART.

among the true Ferns. It has, however, been already mentioned that *Myelopteris* has been found to be the petiole of *Medullosa*, a most characteristic member of the *Cycadofilices*, and this important discovery opens up the whole question of the affinities of *Neuropteris*, and has given rise to the opinion that it is also one of the large though not very well defined group of the *Cycadofilices*,* and this view of the affinities of *Neuropteris* is now confirmed by the discovery of fruiting specimens with the pinnules of *Neuropteris heterophylla*, BRONGT., still attached to the pedicel which bears the seed.

In 1887 I described a small fertile specimen of *Neuropteris heterophylla*, BRONGT., which was discovered by Mr. T. STOCK, in the Lower Coal Measures of Blairpoint, Dysart, Fife.† This specimen (Text figure 1) shows an axis about 8 centims. long, which gives off lateral pinnæ. Both the pinnæ and terminal portion of the axis end in slender naked dichotomously divided branchlets, terminated by an imperfectly preserved small body, which appears to be split into four arms or valves, but the structure of these terminal bodies cannot be clearly made out. This specimen, except in showing that this fertile portion was deprived of the ordinary foliage leaves (though these are borne below the fertile part and allowed of a satisfactory identification of the species), only indicated the mode in which the fructification was produced. Although the significance of this example could not be discovered when it was originally described, in the light of the fruiting specimens of *Neuropteris heterophylla* that have recently come into my hands (and which were collected from the "10 feet Ironstone Measures," which form the roof of the Thick Coal at Clays Croft Openwork, Coseley, near Dudley, and communicated to me for examination by Mr. H. W. HUGHES, F.G.S., Dudley), I think that there cannot remain any doubt that the Blairpoint specimen of *Neuropteris heterophylla* exhibits the pollen-bearing organs of the species.

Among the specimens which were submitted to me for examination from Coseley, which are preserved in small ironstone nodules, were three examples of a *Rhabdocarpus*, each attached to a fragment of a pedicel bearing the foliage of *Neuropteris heterophylla*, BRONGT.

The specimens are shown on Plate 1, figs. 1-9.

* See SCOTT, 'Studies in Fossil Botany,' p. 374, *et seq.*, 1900.

† "On the Fructification of some Ferns from the Carboniferous Formation," 'Trans. Roy. Soc. Edin.,' vol. 33, p. 150, Plate 8, fig. 7.



Fig. 1. — *Neuropteris heterophylla*, BRONGT., showing "male inflorescence." Nat. size. (In collection of Geol. Survey of Scotland, Edin.)

Figs. 1 and 9 are the two halves of the same nodule, shown natural size. Fig. 2 is the specimen shown at fig. 1, enlarged two times. Of the specimen shown natural size at fig. 3, there was only one-half preserved, and this is given magnified two times at fig. 4. Figs. 5 and 7 show the two halves of the same nodule, natural size, and these are shown magnified two times at figs. 6 and 8.

The seeds are all about 3 centims. long; probably the specimen shown at figs. 1 and 9, if it had been perfect in the upper part, would have been slightly longer; their width varies from about 1.10–1.40 centims. Their general form is oblong, but they are gradually contracted from about the middle into a somewhat sharp point.

The outer surface shows numerous longitudinal ribs, formed by bands of sclerenchymatous tissue in the outer envelope of the seed.

On the specimen shown at figs. 1, 2, and 9, the apex is truncate, but this is the result of imperfect preservation, the apex having been broken off. The true form of the seed is seen at figs. 3–8, where the apex forms a micropylar point. The example seen at figs. 1, 2, and 9 is uncompressed, and shows that the seeds were circular in transverse section.

These seeds, if separated from the foliage, would find a place in the genus *Rhabdocarpus*,* and differ little from *Rhabdocarpus tunicatus*, as figured by RENAULT,† or *Rhabdocarpus subtunicatus*, as figured by GRAND'EURY,‡ except in being narrower in proportion to the length.

The internal organisation of *Rhabdocarpus* has been described by BRONGNIART§ and RENAULT.|| It belongs to the *Radiospermeæ* group of the "older Gymnosperms,"¶ but differs much both in size and type of structure from that met with in *Lagenostoma Lomaxi*, the seed of *Lyginopteris Oldhamia*.**

The examples given at figs. 2 and 4 (specimens, figs. 1 and 3 ($\times 2$)) show fragments of the pedicel—in these cases probably the termination of pinnæ—but the rachis is much thicker than it would be in ordinary barren pinnæ, a necessity for the support of so large a seed. Two pinnules are seen attached to the rachis at *a*, fig. 2, and one

* GÖPPERT and BERGER in Berger, "Fructibus et seminibus," p. 20, Vratislavia, 1848.

† RENAULT, 'Flore foss. Terr. houil. d. Commeny' (Part II.), p. 638, Plate 72, fig. 19, 1890.

‡ GRAND'EURY, 'Geol. et Paléont. du bassin houil. du Gard,' p. 328, Plate 6, fig. 6, 1890.

§ BRONGNIART, 'Recher. sur les graines fossiles silicifiées,' pp. 21, 23, Plates 9–12, 1881. *Note*.—BRONGNIART ascribes to *Rhabdocarpus* characters not included in the original description of the genus. BRONGNIART's *Pachytesta*, as judged by external characters, would also find a place in *Rhabdocarpus* G. and B., thus, according to the Brongniartian view, the seed of *Neuropteris* should perhaps be placed in *Pachytesta*.

|| RENAULT, 'Cours d. Botan. Foss.,' vol. 1, p. 110, 1881; also 'Bassin houiller et permien d'Autun et d'Épinac,' fasc. IV.; 'Flore Foss.,' Deux. partie, pp. 387–8, 1896.

¶ See OLIVER, "The Ovules of the Older Gymnosperms," 'Ann. of Botany,' vol. 17, No. LXVII., p. 451, 1903.

** OLIVER and SOOTT, "On *Lagenostoma Lomaxi*, the Seed of *Lyginodendron*," 'Roy. Soc. Proc.,' vol. 71, p. 477, 1903.

at *a* on fig. 4. These do not differ in form or nervation from the ordinary foliage pinnules of *Neuropteris heterophylla*. Figs. 6 and 8, enlargements of figs. 5 and 7, also show a fragment of rachis. The pinnule shown at *a*, fig. 8 ($\times 2$) is not seen on the other half of the nodule given at fig. 6 ($\times 2$). This pinnule, fig. 8 (*a*) is also typical of the foliage of *Neuropteris heterophylla*. That shown at (*a*), fig. 6, shows a slight widening of the base, and seems to be attached to a basal expansion somewhat of the nature of a cupule. This semi-cupule-like structure is better seen at (*b*), especially on fig. 8 ($\times 2$). It does not seem to form a complete cupule surrounding the seed as in *Lagenostoma Lomaxi*, but is more in the form of a subtending tract, and probably served as a protection to the seed during the early stages of its development.

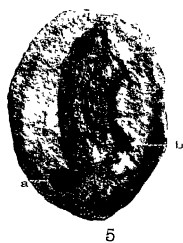
The main rachis of *Neuropteris heterophylla* bore many large Cyclopteroid pinnules,* whose function, the protection of the young growing points, was similar to that performed by *Aphlebia* on *Dactylothea plumosa*, *ARTIS* sp.,† and other genera.

The interest in the Coseley specimens is threefold. They enable us now, I believe, to explain the specimen of *Neuropteris heterophylla* from Blairpoint, to which reference has been already made. Of this example there cannot be any doubt as to its also belonging to *Neuropteris heterophylla*, BRONGT., as the foliage attached on the lower portions of the pinnæ agrees absolutely with the pinnules of that species, and though one cannot decipher the minute structure of the bodies which terminate the naked slender dichotomous branchlets, the occurrence of two forms of fructifying organs on the same species seems to preclude the possibility of doubting that in the Blairpoint specimen we see the remains of the organs which produced the necessary pollen grains for the fructification of the *Rhabdocarpus* seed, and it is further seen that at least some of the numerous seeds referred to the genus *Rhabdocarpus* belong to the Cycadofilices.

The Blairpoint and Coseley specimens also afford the first case where the male and female organs of a single species are known which belongs to that large and important group of carboniferous plants now included in that somewhat undefined assemblage which we call the Cycadofilices.

* ROEHL, 'Fossile Flora d. Steinkohlen-Formation Westphalens,' p. 37, Plate 17 (*Neuropteris Loshii*, BRONGT. = *Neuropteris heterophylla*, BRONGT.).

† L. and H., 'Fossil Flora,' vol. 2, Plates 100 and 101 (*Sphenopteris crenata* and *Schizopteris adnascens* = *Dactylothea plumosa* ARTIS, sp.). KIDSTON, 'Trans. Roy. Soc., Edin.,' vol. 38, p. 216, Plate 2, figs. 6-7 (1896).



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MASSEZ, Geo. Phil. Trans., B, vol. 197, 1904, pp. 7-24.

II. On the Origin of Parasitism in Fungi.

By GEORGE MASSEE, F.L.S., Principal Assistant, Herbarium, Royal Botanic Gardens, Kew.

Communicated by Sir WILLIAM THISELTON-DYER, K.C.M.G., C.I.E., F.R.S.

Received January 11,—Read February 4, 1904.

RESPECTING the various modes by which parasitic fungi gain access to the interior of the host-plant, much is known. DE BARY (1) demonstrated that the germ-tubes of æcidiospores and uredospores enter solely through the stomata, whereas germ-tubes of teleutospores, and also those of various other parasites, enter by piercing the walls of the epidermal cells, or of the guard-cells of the stomata. Other fungi gain an entrance sometimes by a stoma, sometimes by piercing the wall of an epidermal cell.

The same author also observed that the zoospores of *Cystopus* and of *Peronospora umbelliferarum*, when deposited on the leaf of a suitable host-plant, germinate and the germ-tube enters a stoma, whereas when germination takes place in water the germ-tubes soon die. MARSHALL WARD has shown (2) that in the case of a species of *Botrytis* an entrance into the host-plant through the cell-walls of the epidermis is effected by means of the secretion of a ferment by the tip of the germ-tube, whereby the substance of the cell-wall is softened.

On the other hand, but little has been accomplished bearing on the question as to why certain plants are attacked by parasitic fungi, and what has been done turns mainly on supposed special susceptibility, predisposition to disease, or the gradual lowering of the constitution due to cultivation, &c., of the plants attacked.

Such statements, however, even if true in a general sense, do not indicate the specific reason why a parasitic fungus possesses the power of gaining an entrance into the tissues of one particular kind of plant only. Nevertheless, such is well known to be the case, for although the spores of a parasitic fungus will germinate on the surface of the leaf of any plant if it is damp, yet the germ-tubes will only enter the tissues and infect the particular kind of plant on which the fungus is known to be parasitic.

This condition of things suggests the existence of some attractive agency exercised unconsciously by the host-plant over its parasite. This power of the host-plant over

its parasite I shall endeavour to prove to be due to what PFEFFER has called "chemotaxis," or the direction of movement due to chemical stimuli.

The idea that chemotaxis may play some part in favouring parasitism has already been suggested by MARSHALL WARD, who, in discussing predisposition and immunity in plants, says (3): "Moreover, as we know from the recent advances in the study of enzymes and of chemotactic substances, of toxins and antitoxins, such substances and conditions as I have postulated above do actually occur in the living cells, and there is not only no absurdity, but, on the contrary, every show of probability that—since the structural features elucidated by the microscope are not responsible for the phenomena of immunity and susceptibility on the part of the host, or of capacity or incapacity to infect on the part of the fungus—it is in the domain of the invisible biological properties of the living cell that we must expect the phenomena to reside."

ISTVANFFI also considers that chemotaxis may prove to be an important factor in connection with parasitism in fungi (4).

At this stage it may be well to briefly indicate the significance of chemotaxis as defined above. The attraction of plasmodia of the Myxogastres towards a nutritive solution placed in their vicinity was described by STAHL (5), and the term "trophotropism" suggested, but it was due to the brilliant investigations of PFEFFER (6) that the force he termed "chemotaxis" was clearly demonstrated. This author experimented with the antherozoids of cryptogams, bacteria, protozoa, &c., and proved that not only food-stuffs, but also solutions of various salts, acids and alkalies possessed the power of directing the movements of the organisms experimented upon. He further showed that certain substances attracted bacteria, &c.—positive chemotaxis—whereas other substances exercised a repelling influence—negative chemotaxis.

Following PFEFFER, MIYOSHI (7) has shown that by placing the epidermis of an onion bulb-scale or a thin film of mica through which very minute holes had been pierced, on the surface of gelatine containing a positively chemotactic substance, as dextrin, plum decoction, sugar, &c., the germ-tubes of fungus spores sown on the surface of the epidermis or mica grew towards and finally passed through the stomata or pierced holes into the nutrient substance. He also demonstrated that the hyphæ of *Penicillium glaucum* and species of *Botrytis* passed through the epidermal cell-walls of living leaves, which had previously been charged with a positively chemotactic substance by hypodermic injection.

MIYOSHI tested the action of a large number of salts, acids, alkalies, &c., on fungi, and gives the result in tabulated form. Among strongly positive chemotactic substances may be mentioned saccharose, glucose, asparagin and peptone; among the less pronounced in this direction are included levulose and lactose. Maltose is generally neutral. Free organic and inorganic acids, alkaloids and certain salts, proved to be negatively chemotactic or repellent.

The same author also caused the germ-tubes of *Penicillium glaucum* to bore through the cells of elder pith, and to pass through the pits of coniferous wood that had been injected with a chemotactic solution (8). A preliminary series of experiments were first conducted with the object of testing the efficacy of chemotaxis on a larger number of fungi belonging to different groups than had previously been done.

As the primary object of the present research was to discover, if possible, whether the entrance of the germ-tubes of parasitic fungi into the living tissues of a plant was directly due to the attraction of some specific substance present in the cell-sap, only those substances occurring normally in living plants were tested. Among these may be enumerated cane sugar (saccharose), grape sugar (glucose), asparagin, malic acid, and the cell-sap of various plants.

These substances were tested respectively in proportions varying from 0·05–10 per cent., along with 6 per cent. of gelatine. Petri dishes were mostly used for the experiments. A thin sheet of mica or of celluloid of the kind used for photographic films 3 centims. square, having a minute hole pierced through the centre, was placed on the nutrient substance in the petri dish. The upper surface of the mica or celluloid film was moistened with sterilised tap water, and the spores to be experimented upon were sown in the water. A piece of damp blotting paper placed on the inside of the cover of the petri dish prevented desiccation of the spores.

The accepted proof of the germ-tubes of a fungus being chemotactically attracted by the substance present in the nutrient substance, consisted in their growth being directed towards the hole in the mica, and eventually passing down through the perforation into the nutrient substance. When the substance proved to be negatively chemotactic, or repellent, the germ-tubes showed no tendency to grow towards the perforation in the mica, or those that happened to grow in its direction altered their course and receded when they approached within the sphere of its influence.

In other experiments the epidermis of an orchid—*Oncidium bellutulum*—was used instead of mica.

It is important to bear in mind that chemotaxis is not in any way connected with germination. Practically all spores, whether belonging to saprophytes or parasites, will germinate in water alone, or in a dilute solution of sugar in water, without, however, its exercising the slightest influence on the direction in which the germ-tubes grow. It is only when a substance proving to be positively or negatively chemotactic as the case may be, is localised at some given point, that growing germ-tubes are attracted or repelled by its presence, when once they approach within the range of its influence.

In experiments conducted by sowing spores in a film of water on a mica or celluloid plate, placed on a medium to be tested as to its chemotactic power, it may be assumed that a small amount of the substance passes up through the perforation

in the mica plate, and slowly diffuses in the water on its surface. If the substance proves to be positively chemotactic to the fungus experimented with, the germ-tubes on coming in contact with the substance diffused in the water, follow the diffused substance, which becomes more concentrated as the perforation in the mica is approached, through which the germ-tubes eventually pass into the yet denser attractive substance in the gelatine.

If the substance proves to be not chemotactic, the germ-tubes grow in various directions, and are not at all influenced by the substance diffused in the film of water on the mica, and do not pass through the perforation into the gelatine below.

This last experiment proves that difference of density in the absence of a chemotactic substance does not enter into the principles involved in chemotaxis, as the germ-tubes do not pass from the film of water on the surface of the mica, into the denser substance below unless specifically attracted by some other factor than that of difference of density.

Finally, to prove that germ-tubes do not pass through minute openings, simply because such openings in the form of stomata exist in the epidermis of leaves, as appears to sometimes have been assumed; a 2-per-cent. solution of sugar in water was placed in a Petri dish, a plate of celluloid perforated with numerous minute holes was floated on the surface of the solution, and its upper surface moistened with a portion of the solution on which it floated. Spores of *Botrytis cinerea* were sown in the liquid on the surface of the mica, and although germination was very vigorous, the germ-tubes did not pass through the perforations in the mica into the solution below.

MIYOSHI does not give details of his mode of injecting living leaves. The method I employed is as follows: Living leaves still attached to the plant were injected with the chemotactic solution. An ordinary hypodermic syringe having a very fine needle was used. In a fairly fleshy leaf the needle can, with practice, be inserted for a distance of 2 centims., just under the epidermis of the under surface of the leaf. As the needle is very slowly withdrawn, a slight pressure is applied to the piston; when successfully accomplished, up to 0.5 of a cubic centimetre of the solution can be injected, which betrays its presence externally by a slight discoloration of the epidermis, and extends over an area of 1-2 square centims. The spores are sown in a film of water on the surface of the cuticle, over the injected portion.

The following table shows the positive (+), or negative (-) chemotactic action of various substances, on the germ-tubes of fungi. Those fungi marked with an asterisk (*) are obligate parasites; a dagger (†) indicates facultative parasites; the remainder, so far as is known, are pure saprophytes. A 2-per-cent. solution of the substance to be tested, in 6 per cent. of gelatine was used.

A glance at the table shows that sugar is by far the most general of positive chemotactic substances. The germ-tubes of all the saprophytes, with the exception of

Name of fungus.	Cane sugar.	Grape sugar.	Asparagin.	Malic acid.	Decoction of cucumber leaves.	Decoction of tomato leaves.	Decoction of bramble leaves.
* <i>Cercospora melonis</i> . .	-	-	-	-	+	-	
* <i>Macrosporium tomato</i> .	-	-	-	-	-	+	
* <i>Cladosporium fulcum</i> .	-	-	-	-	-	+	
† <i>Botrytis cinerea</i> . . .	+	+	+	-	-	-	
<i>Trichothecium candidum</i>	+	+	-	-	-	-	
† <i>Cladosporium epiphyllum</i>	+	+	-	...	-	-	
† <i>Penicillium glaucum</i> .	+	+	-	-	-	-	
† <i>Trichoderma lignorum</i>	+	+	+	...	-	-	
* <i>Sclerotinia fructigena</i> . (Conidia)	+	+	-	+	-	-	
† <i>Dendryphium comosum</i>	+	+	-	-	+	-	-
† <i>Fusicladium dendriticum</i>	+	-	-	+	-	-	
† <i>Mucor racemosus</i> . .	+	+	-	-	-	-	
<i>Stysanus stemonites</i> .	-	-	-	-	-	-	
<i>Verticillium lateritium</i>	+	+	-	-	-	-	
<i>Torula herbarum</i> . .	+	+	-	-	-	-	
<i>Bispora moniloides</i> .	+	+	-	-	-	-	
* <i>Phragmidium violaceum</i> (teleutospores)	-	-	-	-	-	-	-

Stysanus stemonites, and all the facultative parasites respond strongly to this substance. On the other hand, the action of sugar on the germ-tubes of obligate parasites, although evident, is very slight, and was not sufficiently powerful in any instance to attract the germ-tubes through perforations in mica, or through stomata.

On again referring to the table it will be seen that the only substance exercising a marked positive chemotactic influence over the germ-tubes of obligate parasites, is the cell-sap of the host-plant on which they are parasitic.

This discovery gives us the first glimpse as to why certain fungi are obligate parasites on one species of plant only. It means that the fungus has become so sharply specialised, that its germ-tubes respond alone to a given substance, or to a combination of substances, present only in the cell-sap of one particular species of plant.

When the specialisation of the fungus is not so sharply defined, we find it capable of attacking several allied species of plants; whereas when we come to such facultative parasites as *Botrytis*, which attacks a greater number of plants belonging to widely separated affinities than any other fungus, it is found that the presence of sugar alone in the cell-sap is sufficient to establish parasitism, provided other conditions are favourable.

The reason why *Botrytis* does not become parasitic on every plant containing sugar in its cell-sap, is probably due to the presence of other substances in the cell-sap which are negatively chemotactic to its germ-tubes.

The following experiments support this idea :—

Experiment 1.—When *Botrytis* spores are sown in a decoction of the cell-sap of green apples, the percentage of germination is small, and the germ-tubes do not continue to grow after the third day. When the apple juice along with gelatine, is used as a chemotactic substance, the spores being sown in water on a mica film, its action is negative. The spores will not germinate in a solution of malic acid in water.

Now, although there is sufficient sugar present in the apple cell-sap to constitute a positive chemotactic substance for the *Botrytis* germ-tubes, this action is neutralised by the more powerful negative chemotaxis of the malic acid present. *Botrytis* is not parasitic on green apples in a state of nature.

Experiment 2.—This experiment was conducted after the method described above, the cell-sap of a half-ripe tomato being substituted for that of apple. Germination of *Botrytis* spores was feeble in the cell-sap, and this substance also proved to be negatively chemotactic. On experimenting with oxalic acid, a substance present in tomato fruit, its action proved to be distinctly repellent to the germ-tubes of *Botrytis*. In this instance, therefore, the repellent action of oxalic acid secured the tomato from being attacked by *Botrytis*.

Negative chemotaxis has been shown by ZILBERBERG and ZELIONY (9) to be the cause of immunity against the action of certain virulent bacteria.

Returning to a study of the behaviour of obligate parasites to certain substances normally present in the cell-sap of their respective host-plants, *Sclerotinia fructigena*, the asexual form of which is better known in this country as *Monilia fructigena*, is a well-known destructive parasite on the fruit of apple, pear, plum, cherry, &c.

Experiments conducted with the spores of this fungus, obtained from mummified fruit, and kept for a long time on the ground throughout the winter, showed that the spores were attracted to sugar alone, also to malic acid alone, but are repelled by oxalic acid. They were attracted to 1 per cent. of sugar and 1 per cent. of malic acid, but not to 1 per cent. of oxalic acid. They showed no directive response, however, to a mixture of 1 per cent. of sugar and 1 per cent. of malic acid, either in the form of a solution or in the form of a solid. The normal constituent of apple cell-sap, is the only substance which attracts the germ-tubes of *Monilia* into the tissues of the fruit.

Experiments were also conducted with the spores of *Sclerotinia fructigena*, obtained from fruit which had been kept for a long time on the ground, and which were very slightly, or not at all, attacked by the fungus. These spores showed a very slight attraction with sugar, but no attraction with malic acid, and were repelled by oxalic acid. They showed no directive response, however, to a mixture of 1 per cent. of sugar and 1 per cent. of malic acid, either in the form of a solution or in the form of a solid. The normal constituent of apple cell-sap, is the only substance which attracts the germ-tubes of *Monilia* into the tissues of the fruit.

This idea agrees with the observations of ERIKSSON (10), MARSHALL WARD (3c), and SALMON (11), that biological forms of species of *Puccinia* and *Erysiphe* are restricted to special hosts or even, in some instances, to forms or varieties of a species of host-plant.

The remarks of MARSHALL WARD (3D), respecting the non-adaptability of Uredospores to certain species of *Bromus*, appear to bear on the above subject. He says: "In these cases it seems that the infecting tubes reach and penetrate the stomata but are unable to form a mycelium in the tissues, evidently because the living cells are really resistant. How the latter prevent the hyphæ from putting haustoria into their cell cavities is not yet clear, but it looks as if they exerted some deleterious influence on the delicate tips, and thus brought their efforts to abortion."

The answer suggested by the experiments described in this paper is that the non-adaptability was due to the action of negative chemotaxis.

Cercospora melonis is a very destructive parasite, met with only on species of *Cucumis*. The germ-tubes respond chemotactically only to a solution of the host-plant. Experiments seem to indicate that the enzyme called pectase, which is present in some considerable quantity in cucumber leaves, is the specific chemotactic substance in this instance. On the addition of a watery solution of pectine to the extract of cucumber leaves, the chemotactic property of the sap is destroyed, probably due to the coagulation of the pectine by the pectase. Boiling the expressed juice of cucumber leaves, or the addition of a trace of acetic acid also destroy its positive chemotactic influence over the germ-tubes of *Cercospora*.

Pectase is most abundant in leaves that have grown rapidly, hence the greater susceptibility of cucumber plants that are much "forced" over those growing under more normal conditions.

Teleutospores of *Phragmidium violaceum* germinated in water, also in the expressed juice of bramble leaves, the natural host-plant of this obligate parasite. Some time was spent in endeavouring to discover a positive chemotactic substance for the germ-tubes, but without success, the cell-sap of the host-plant even proving to be inert. Finally it occurred to me that the germ-tubes of teleutospores are not infection tubes, but promycelial tubes, which soon produce a few wind-borne promycelium spores. It is the latter spores that directly enter the tissues and infect the host-plant, but I have not had an opportunity of testing the effect of bramble leaf cell-sap on promycelium spores.

In using the expressed juice of leaves for experimental purposes, percentages as used in the ordinary sense cannot be given, but I have adopted the following standard:—Fourteen grammes of the blade of the leaf are thoroughly crushed in a mortar, along with 4 c.c. of sterilised water. The pounded mass is squeezed through muslin, and the liquid product constitutes the stock solution.

This solution is only effective when used immediately after preparation. Boiling destroys its chemotactic property, and the addition of a trace of acetic acid, added

with the idea of checking the development of bacteria, also rendered it neutral to germ-tubes.

A 2-per-cent. of the stock solution prepared from cucumber leaves proved strongly positively chemotactic to the germ-tubes of *Cercospora melonis*, whereas a 5-per-cent. proved as decidedly negative.

Professor MARSHALL WARD has kindly pointed out to me that the expressed juice of a leaf is a very different thing from the cell-sap of the same leaf. Expressed juice is certainly an unknown quantity, and unfortunately the same may be said of cell sap to a great extent. I have not always been able to detect the exact substance or combination of substances present in the cell-sap, which determines its chemotactic property. In most instances the components of the cell-sap are unknown, and even where they are known are difficult to procure. This difficulty will, however, in all probability be eventually overcome.

At present, however, it is something to know that when a positive chemotactic substance is present in leaf extract, its efficacy is not obliterated if used when quite fresh.

The spores of *Cercospora melonis* germinate freely in 3-per-cent. gelatine and 2-per-cent. sugar, but the germ-tubes show not the slightest directive influence, whereas if the proper amount of cucumber-leaf decoction is added, the germ-tubes are strongly attracted. The same is true of the germ-tubes of other parasites.

MIYOSHI (7A) found that the germ-tubes of *Uredo linearis* were attracted by a decoction of wheat leaves, the host-plant of this parasite, and further demonstrated that the germ-tubes of this fungus, when sown on the epidermis of a leaf of *Tradescantia discolor* that had been injected with a decoction of wheat leaves, passed through the stomata into the tissues of the leaf.

RAY (12) in attempting to convert such sharply defined obligate parasites as species of the Uredinæ and the Ustilaginæ to a saprophytic condition, discovered that to accomplish this object, the fungi had first to be cultivated in a decoction of the host-plant on which they were respectively parasitic, and replacing this nutrient medium by degrees with decoctions of plants less and less allied to the original host.

The experiments described I consider to point to the conclusion that parasitic fungi, and more especially obligate parasites, are highly specialised forms, whose germ-tubes have gradually acquired the property of responding chemotactically to one specific substance only, other than sugar, and even the last-named substance, as previously stated, also plays a certain part in the work of enabling the fungus to enter its host; possibly this part may be connected with the nutrition of the germ-tubes. In the case of the less highly specialised facultative parasites, it would appear that sugar alone is sufficient to enable the germ-tubes to enter the tissues of a plant, unless its power is neutralised by the presence of a more potent negatively chemotactic substance.

It is generally considered that infection of plants by parasitic fungi occurs most frequently during the night in a state of nature. The following experiment supports this idea:—

A batch of vegetable marrow plants growing in my garden last year were badly attached by *Sphaerotheca humuli*. Twelve young leaves showing no trace of the disease were selected for experiment. Six leaves were protected during the day (6 A.M. to 6 P.M.) in paper bags, and left exposed during the night. Six other leaves were enclosed in bags during the night (6 P.M. to 6 A.M.) and exposed during the day. This arrangement was continued for a week; those leaves that had been exposed during the night only were white with the mildew at the termination of the experiment, whereas those leaves that had been exposed during the day only were free from the disease, with the exception of a few very small patches on three of the leaves.

Some of the causes favouring infection during the night are as follows: (1) The presence of a film of moisture on the surface of leaves, &c., an indispensable factor. (2) A greater amount of sugar in the cell-sap. (3) Perhaps a greater amount of those special substances which prove positively chemotactic to the germ-tubes of the parasite; on this point, however, I can offer no direct evidence.

The above conditions collectively tend to produce temporarily, in the plant growing in the open, the "soft" foliage of the gardener, a condition which all experience has proved to be highly susceptible to disease.

In plants grown under glass, more especially when "forced," the conditions favouring "soft" foliage are constantly at work; which in the aggregate mean the loss of balance between work done by leaves and root respectively. Hence the reason why plants grown under glass are so much more susceptible to disease, than are plants of the same kind grown out of doors.

MARSHALL WARD points out (3E), that if a period favourable to growth of plant life is followed by a fall of temperature, rain, and obscured light, transpiration is lowered and the plant becomes more suffused with water, hence turgescence is promoted. Under such conditions the absorption of oxygen is decreased, and not present in sufficient quantity to complete the oxidations and decompose the organic acids, which accumulate in the cells; such bodies accompanied by glucose in solution, predispose a plant to disease.

Speaking of the potato disease, the last quoted author says (3F).—"Suppose we take a potato plant the leaves of which are very slightly marked with disease spots, and divide it into two halves as exactly alike as possible, and place each half in a tumbler of water; the two tumblers with their half-plants, are then placed in an ordinary room, side by side, at a temperature of about 20° C., and one is covered close with a bell-jar, and the other left uncovered. In a short time—often a few hours—the covered leaves become black and rotten with the disease; whereas the

uncovered one will go on looking fresh for several days, though it also succumbs at once if covered.

"The question arises whether the rapid spread of the fungus and the rot it causes here are simply owing to the increased supply of water, as the tissues become turgid in the saturated atmosphere under the bell-jar; or whether we have not here again, in addition, a case where the diminished access of oxygen to the interior of the tissues of the host results in an accumulation of organic acids and other substances which make the excessively turgid cells and thin watery cell-walls more than usually easy prey to the parasite."

The researches of PFEFFER, MIYOSHI, also my own investigations bearing on chemotaxis, prove that in some instances a very small percentage of a chemotactic substance is sufficient to clearly indicate its positive or negative property. Thus 0.01 per cent. of most organic acids and alkalis is decidedly negative to the germ-tubes of many fungi, whereas the same percentage of cane sugar is as markedly positive in its action. Many substances, as sugar and asparagin, exhibit distinctly positive chemotropic properties in low dilutions, whereas the same substances in higher dilutions, exhibit negative chemotaxis.

It is possible that the prime factor in determining the presence or absence of an epidemic caused by fungi, may not depend so much upon the superabundance of fungus spores in the first instance, as upon the exact amount of chemolactic substance present in the cell-sap of the host-plant; assuming that the amount of such substance is variable, even within very narrow limits, under varied climatic or other conditions.

A further corroboration of the view that parasitism on the part of fungi, is determined by the presence of some attractive substance in the cell sap, was furnished by experiments made on an immune cucumber plant. Up to the present, the conception of an immune plant is an individual that resists the attacks of a given parasite, without, however, being able to assign a definite reason as to why; and even in the most virulent epidemics, it is not unusual to find certain individuals possessing this property of immunity.

The plant experimented upon was one of a batch of ten, brought to Kew by a market gardener from Waltham Cross, whose plants had suffered severely from an epidemic during the previous season. All the plants were supposed to be attacked by a fungus *Dendryphium comosum*, and were placed in an experimental house for future observation. One of the plants, however, proved to be free from disease, and although surrounded by diseased plants for months, this plant resisted inoculation, both from natural and artificial sources.

Numerous other cucumber plants were successfully infected with spores from the same gatherings as those experimented with on the immune plant. On sowing spores on mica films, in a 2-per-cent. infusion of the cell-sap of the immune plant, I was surprised to find that the percentage of spores that germinated was small, and

that the germ-tubes did not respond chemotactically to the nutrient substance. Numerous further experiments were made with dilutions of the cell-sap of the immune plant, varying from 0·05–5 per cent., and under different temperatures ranging from 50–80° F., but the percentage of germination was in all instances low, and the germ-tubes absolutely refused to be influenced in their direction by the cell-sap.

Check experiments with spores sown on mica films, in contact with a 2-per-cent. solution of cell-sap, obtained from the leaves of susceptible, or in other words, diseased cucumber plants, germinated freely, and the germ-tubes were strongly chemotactic.

These experiments, coupled with others made on immune tomato plants, using spores of *Macrosporium tomato* for the purpose, prove that in future we shall be justified in defining an immune plant as an individual, in which the positive chemotactic substance, necessary for facilitating the entrance of the germ-tubes of a given parasitic fungus into its tissues, is absent.

The endeavour to produce immune races of economic plants has been much prosecuted of late years, and a certain amount of success has in some instances attended such endeavours; but apart from the fact that immune plants have constituted the basis of such experiments, the points aimed at have been the production of a more resistant cuticle, bloom on the leaves, &c.

Now, with clearer knowledge as to what constitutes immunity, it follows that the modifications required to attain this desired object are physiological rather than morphological; and the first step must necessarily be the determination of the exact constituent or constituents of the cell-sap, exercising a chemotactic influence over the germ-tubes of parasitic fungi.

MARSHALL WARD has demonstrated (3A), that the respective susceptibility or immunity of species of *Bromus*, to the attack of Uredospores of *Puccinia dispersa*, is not at all influenced by morphological characters. His summary on this point is as follows:—"The capacity for infection, or for resistance to infection, is independent of the anatomical structure of the leaf, and must depend on some other internal factor or factors in the plant."

The method of procedure which has resulted in demonstrating the possibility of inducing saprophytic species of fungi to become parasites, or of educating a fungus already a parasite, to change its host-plant, is again only an extension of the system followed by MIYOSHI in studying chemotaxis, or as he writes it, "chemotropism."

This author, among other experiments, sowed spores of *Penicillium glaucum* on the surface of a leaf of *Tradescantia discolor* into the tissues of which a 2-per-cent. solution of cane sugar had been injected. Not only did the germ-tubes of the *Penicillium* pass through the epidermis, but the mycelium spread vigorously and showed a tendency towards parasitism in the tissues of the leaf.

It is important to note that this tendency towards parasitism was manifested by *Penicillium* during the single experiment conducted by MIYOSHI. This evidence

suggested the idea that if the spores produced by a fungus showing a parasitic tendency, were again used for infecting a similar kind of host-plant, the incipient parasitism might gradually become intensified, and experiments about to be described proved this idea to be correct.

Experiments were conducted with the following species of fungi, which happened to be available at the time. *Trichothecium candidum*, *Torula herbarum*, *Stachybotryum lobulatum*, and *Stysanus stemonites*. These all belong to the Hyphomycetes and so far as is known are pure saprophytes, growing on decaying plants, damp paper, dung, &c. Spores of these fungi were first sown on perforated films of mica placed on gelatine containing 2 per cent. of a solution of cane sugar. The germ-tubes of *Stemonites* were not attracted by the sugar solution, hence this species was omitted from further experiments.

Six sowings of each of the three remaining species were made on living leaves still attached to the plant, *Begonia kewensis*, which had previously been injected with a 2-per-cent. solution of cane sugar in water. The spores were sown in a film of water on the surface of the leaf over the portions injected with sugar. An equal number of check sowings were made at the same time, on leaves not injected with a sugar solution.

Five days after sowing the spores, two out of the six patches infected with *Trichothecium candidum* each showed a few erect hyphæ, bearing spores, and microscopic examination showed that these hyphæ emerged from the interior of the plant through the stomata. These spores were at once sown on *Begonia* leaves injected with sugar solution as before, and this method of sowing spores produced by the preceding generation was repeated fifteen times. From four to five of the six patches sown produced spores during the last six batches of experiments, proving that the fungus was becoming more and more accustomed to its new environment.

When the quantity of spores produced from leaves injected with sugar solution became fairly abundant, some of these were sown on *Begonia* leaves that had not been injected with a solution of sugar; and from a sowing of the twelfth generation of spores produced on injected areas, but sown on a leaf not containing an injected chemotactic substance, I was delighted to see, the fourth day after sowing the spores, a small crop of sporophores bearing spores. This means that after twelve generations of the fungus, educated to grow in living *Begonia* by means of a chemotactic substance—a solution of cane sugar—the faculty of parasitism had been acquired for this particular host-plant.

Spores obtained from the thirteenth and fourteenth generations grown on injected areas showed more vigorous parasitism; whereas spores from the fifteenth and last crop of spores produced from injected areas, proved to be vigorously parasitic when sown in a film of pure water on *Begonia* leaves not injected with a solution of sugar.

The period of time required in this series of experiments for the conversion of a

saprophytic fungus into a true parasite was twelve weeks, and the number of generations of the fungus amounted to sixteen. The host-plant during the experiments was growing in a house where the day temperature ranged between 70° and 80° F., falling during the night to 60° F.

The actual proofs that the *Trichothecium* had become a true parasite on the *Begonia*, consisted in the presence of a copious development of mycelium in intercellular spaces of the leaf, and the occurrence of numerous short, special branches or haustoria in the cell cavities; and also the fact that specialised branches or sporophores, originating from the intercellular mycelium, passed through the stomata and produced fruit in the air. The portions of the leaf infested with the fungus eventually sunk below the general level of the surface, became brown, and the cells collapsed.

The check sowings of spores on *Begonia* leaves not injected, always ended without showing any appearance of parasitism, and never produced fruit; neither could I discover any traces of mycelium in the tissues of the leaf at these points where the spores were sown.

Returning to the results of the experiments made with the spores of *Stachybotryum lobulatum* and *Torula herbarum*. No signs of injury to the host-plant followed sowings of spores of the first-named, and no trace of mycelium was detected in the tissues, nevertheless the spores germinated readily in a hanging drop consisting of a 2-per-cent. solution of cane sugar. It is obvious that for some unknown reason, the germ-tubes of *Stachybotryum* could not effect an entrance into the *Begonia* leaf.

With *Torula herbarum* the case was very different; those areas of the leaf over which the spores were sown soon showed signs of internal disturbance; in fact, these symptoms were more pronounced than in the case of *Trichothecium*, and sections of the leaf showed a fair development of mycelium in the intercellular spaces. Nevertheless the fungus never produced fruit on the surface of the leaf over the infected areas, and this I consider as the most important evidence of parasitism. The results were as described above in twelve sowings, out of which seven showed indications of mycelium in the tissues. In one example where the appearance of the infected area indicated special activity of mycelium in the leaf, I tore a small slit in the epidermis with a fine needle, and at the expiration of two days a copious growth of spores was produced in the opening made in the epidermis.

These spores, as soon as their power to germinate had been determined, were sown on injected patches of *Begonia* leaves, but the result was as previously described, several of the patches showed evidence of the presence of living mycelium in the interior of the leaf, but no spores were developed.

Experiments made with another Hyphomycete, *Zygodesmus fuscus*, gave results similar to those of *Torula*; evidences of parasitism followed the sowing of the spores, but in no instance were spores produced. On the other hand *Cladosporium epiphyllum* spores, when sown on *Begonia* leaves under the conditions already

described, eventually became parasitic, and produced fruit on the surface of the leaf, the sporophores pushing through the stomata from the interior. In this instance, however, the parasitism was very weak, compared with that of *Trichothecium candidum*.

The reason why one species of saprophytic fungus can be induced to become parasitic on a given host-plant, whereas another species treated in every respect in a similar manner fails to do so, I have not been able as yet to determine. The two species that eventually became genuine parasites, *Trichothecium candidum* and *Cladosporium epiphyllum*, agree in having comparatively elongated sporophores, which grow erect from the prostrate vegetative mycelium. Now it is just possible that if one of these sporophores originated from mycelium located in the large intercellular space underlying a stoma, the innate tendency to grow erect might be sufficient to enable it to push through the stoma. On the other hand, the two species of fungi that failed to become parasitic, bear their spores on exceedingly short sporophores that do not grow erect or away from the mass of vegetative mycelium, hence their inability to reach the surface of the leaf. It would probably require a long experience to enable such fungi to form their spores in the substance of the leaf, and eventually rupture the epidermis for the purpose of spore dispersion, after the fashion followed by members of the Uredineæ, even if it could ever be acquired.

A further set of experiments conducted exactly on the lines already indicated, but using an infusion of cucumber leaves instead of a solution of cane sugar for injecting, proved that after twenty-one generations *Cercospora melonis*, an obligate parasite on plants belonging to the genus *Cucumis*, so far as is at present known, was trained to become a true parasite on *Oncidium bellatulum*, a plant belonging to the Orchidaceæ.

It will be observed that a greater number of generations, implying a longer period of time, was required to educate a fungus, already a parasite on one particular plant, to become parasitic on another plant belonging to a family widely separated from the one on which it was originally parasitic than to train a pure saprophyte to become a parasite. The only suggestion I can offer on this point is that the obligate parasite is much more dependent on one particular element for its development than is the case with a saprophyte. The explanation however, I am afraid, cannot be considered altogether satisfactory, inasmuch as at least many saprophytes are also obligate, or, in other words, are mainly if not entirely met with on one particular kind of nutritive pabulum.

It may be argued that the method of injection described above wounds the host-plant, and that the fungus grows as a saprophyte on the wounded portion of the leaf. The fact that the fungus experimented with under such conditions eventually becomes a parasite, whereas spores of the same fungus sown on leaves of the same plant, not injected with a chemotactic substance, are not capable of entering the

tissues, proves, I consider, that the injected substance, to which the fungus already responds, merely serves the purpose of enabling the fungus to become accustomed by degrees to a new nutritive and chemotactic substance present in the living tissues of the plant; when this acquirement on the part of the fungus is complete, parasitism results.

The experiments described above suggest the idea that an increase of virulence on the part of a parasite is possible by cultivation. This possibility, or even probability, I consider to be greatly influenced by circumstances. Last year the very destructive epidemic among cucumber plants, which resulted in a loss of over £20,000 to the growers of these plants in the south of England, was entirely due to the fungus called *Cercospora melonis*.

This spring a market gardener, whose cucumbers had suffered severely from the *Cercospora* last season, brought some cucumber plants to Kew, which he was afraid were attacked by the same disease. Examination showed that the plants were not attacked by *Cercospora melonis*, but by a fungus called *Dendryphium comosum*. The injury caused by the *Dendryphium* has now assumed the proportions of an epidemic in the cucumber houses of this particular market gardener, but is not recorded under such circumstances from elsewhere. Now *Dendryphium comosum* is a well-known saprophyte, growing on decaying vegetable matter, but its occurrence as a parasite has never before been recorded.

A careful examination as to the origin of the outbreak of the epidemic caused by *Dendryphium* revealed the fact that the young cucumber plants were growing in a mixture of two thirds loam and one-third of stable manure. Further, the portions of half-decomposed straw from the manure that projected above the surface of the soil, were covered with a copious growth of *Dendryphium*. The fungus had been introduced with the manure.

Dendryphium spores from the manure, when placed in a drop of water on the under surface of living cucumber leaves, produced the disease. The germ-tubes of the spores were positively chemotactic to a decoction of expressed juice from cucumber leaves, but failed to respond chemotactically to the expressed juice of twelve other plants selected at random and representing seven natural orders.

The above I consider to demonstrate the presence of a latent power—that of being positively chemotactic to a given substance or combination of substances—possessed by a saprophytic fungus. In the present instance the *Dendryphium* happened to be brought in contact with a living plant possessing the chemotactic influence required to enable the fungus to become a parasite.

A second illustration of extended parasitism, due entirely to opportunity, is afforded by *Puccinia malvacearum*. This fungus was first observed in Britain in 1873, on cultivated hollyhocks. Since that date the fungus has attacked all our indigenous malvaceous plants included in the three genera, *Malva*, *Aithæa*, and *Lavatera*. It has also occurred on species of *Abutilon* in greenhouses,

It would be interesting to determine whether the forms of this fungus growing on different genera have become "biologic species" within 30 years.

The above conclusions are simply the reversion of experiments conducted by RAY (12), who in his studies on the biology of parasitism, considered the first important step to be that of educating obligate parasites to become saprophytes. This was accomplished by first growing such fungi in a decoction of the plant on which they were parasitic, followed by cultures in a decoction of a variety of the host-plant, then in that of allied species of plants; thus by a simultaneous process of weaning the fungus of its original food, and accustoming it to a new diet, such pronounced parasites as species of *Ustilago* and *Puccinia* were eventually trained to grow vigorously as pure saprophytes.

The experiments described above I consider to prove, what has previously only been assumed, that parasitism in fungi is an acquired habit.

In experimenting with comparatively large living plants the risk of contamination is great, and in spite of all precautions the cultures are too frequently ruined by the growth of saprophytic fungi such as *Mucor*, *Penicillium*, &c. Before injecting with the chemotactic solution the entire plant, soil, and plant-pot were thoroughly sprayed with a solution of permanganate of potassium. The plant-pot was placed on a sheet of glass covered with damp sterilised blotting paper and covered with a bell-jar also sterilised inside. Through a small opening at the top of the bell-jar water could be conveyed to the plant through a glass tube, and the nozzle of a small sprayer, of the kind used for fixing pencil drawings, could also be introduced, and the plant and inside of the bell-jar sprayed from time to time, with permanganate of potassium solution, which answered the double purpose of arresting the growth of stray fungi, and of keeping the atmosphere damp. As the spraying was directed from above, only the upper surface of the leaves was covered with the fungicide, and this did not interfere with the injected areas on the under surface of the leaves. The experiments described above were conducted in the Jodrell Laboratory, Royal Botanic Gardens, Kew.

In conclusion, it may be well to call attention to the parallelism between the germ-tubes of fungi and pollen-tubes. MIYOSHI (7A) has proved that pollen-tubes show marked positive and negative chemotactic properties; this may account for the behaviour of pollen when placed on the stigmas of flowers other than its own species, and may possibly be the determining factor in the production of hybrids. MARSHALL WARD (8a) gives an exceedingly interesting account "of some remarkable resemblances or coincidences, between the behaviour of these fungus-spores towards their host and, reciprocally of the host to the parasite, and that of the pollen and stigma the one towards the other."

I take this opportunity of thanking Professor MARSHALL WARD and Professor J. RAVENHILL for valuable criticism and suggestions in connection with the present research.

Summary.

1. The entrance of the germ-tubes of a parasitic fungus into the tissues of a living, healthy plant depends on the presence of some substance, in the cells of the host, attractive to the fungus. In other words, infection is due to positive chemotaxis.

2. A saprophytic fungus can be gradually educated to become an active parasite to a given host-plant, by means of introducing a substance positively chemotactic to the fungus into the tissues of the host. By similar means a parasitic fungus can be induced to become parasitic on a new host.

3. An immune plant signifies an individual of the same species as the one on which a given species of fungus is parasitic; but which, owing to the absence of the chemotactic substance in its tissues necessary to enable the germ-tubes of the fungus to penetrate, remains unattacked.

BIBLIOGRAPHY.

1. DE BARY, A. "Fungi, Bacteria, and Mycetozoa" (Engl. ed.), pp. 361-362 (1887).
2. WARD, H. MARSHALL "A Lily Disease." 'Annals of Botany,' vol. 2, p. 319 (1888).
3. ———— "On the Question of 'Predisposition' and 'Immunity' in Plants," 'Cambridge Phil. Soc. Proc.,' vol. 11, p. 326 (1902).
- 3A. ———— *Ibid.*, p. 323.
- 3B. ———— *Ibid.*, 326-328.
- 3C. ———— 'Annals of Botany,' vol. 16, pp. 298-299 (1902).
- 3D. ———— *Ibid.*, p. 298.
- 3E. ———— "Croonian Lecture." 'Roy. Soc. Proc.,' vol. 47, p. 418 (1890).
- 3F. ———— *Ibid.*, p. 437.
4. ISTVANFII, GY. DE. "Études sur le Rot livide de la Vigne." 'Ann. Inst. Ampél. Roy. Hongrois,' vol. 2, pp. 195-196 (1902).
5. STAHL, E. "Zur Biologie der Myxomycetes." 'Bot. Ztg.,' pp. 161-165 (1884).
6. PFEFFER, W. "Locomotorische Richtungsbewegungen durch chemische Reize." 'Ber. d. Deutsch. Bot. Gesell.,' vol. 1, p. 524 (1883); also "Locomotorische Richtungsbewegungen durch chemische Reize." 'Unters. Bot. Inst. zu Tübingen,' vol. 1, p. 363 (1884); also "Über chemotaktische Bewegungen von Bacteria, Flagellaten und Volvocineen," *Ibid.*, vol. 2, p. 582 (1888).
7. MIYOSHI, M. "Ueber Chemotropismus der Pilze." 'Bot. Ztg.,' p. 1 (1894).
- 7A. ———— *Ibid.*, p. 24.

8. MIYOSHI, M. "Die Durchborung von Membranen durch Pilzfaden." 'Pringsh. Jahrb.,' vol. 28, p. 269, (1895).
9. ZILBERBERG, A. and ZELIONY, J. 'Ann. Inst. Past.,' vol. 15, p. 615 (1901).
10. ERIKSSON, J. "Die Getreideroste," p. 108 (1896).
11. SALMON, E. S. "On Specialisation of Parasitism in the Erysiphaceae," 'Beihefte z. Bot. Centralbl.,' vol. 14, p. 261 (1903).
12. RAY, J. "Etude biologique sur le Parasitisme." 'Compt. Rend.,' vol. 136, p. 566 (1903).

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III. *On the "Islets of Langerhans" in the Pancreas.**

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i. *Introductory.*

THE observations here described are the outcome of an investigation of the histological changes produced in the pancreas by the activity called forth by "secretin" (BAYLISS and STARLING (1)). In all former investigations of these changes the secretion of the gland has been provoked by the natural stimulus of digestion or by the administration of pilocarpine. The former is at best a slow stimulus, and the histological variations at different periods of normal digestion slight; the latter was found in practice much inferior to secretin as a pancreatic stimulant, judging by the volume of juice secreted. It seemed worth while, therefore, to repeat the former histological observations with the use of secretin,

* The expenses of this research have been in part defrayed by a grant from the Government Grant Committee of the Royal Society.

which, besides being a far more powerful stimulant than pilocarpine, has the advantage that its action is an intensification of the normal physiological stimulus. It may be said at once that, in regard to the cytological details of the changes involved in the actual secretion—the discharge of the zymogen granules and the growth from the base of the cell of the chromatophilous substance—nothing new was observed. The changes were in many cases more complete, but of the same kind as those described as a result of the action of pilocarpine. My attention, however, was early drawn to very obvious differences produced in the structures called "islets of Langerhans," "intertubular cell-clumps," &c. These changes form the main subject of this paper, and a short historical account seems called for of the views which have been held as to the nature and function of these structures, which will be here referred to as "islets of Langerhans" or "islets."

ii. *Historical.*

LANGERHANS (2), in 1869, first described, in the pancreas of the rabbit, certain roundish areas of tissue, varying in diameter between 0.1 and 0.24 millim., regularly distributed among the ordinary alveoli, but staining a deep yellow with MÜLLER'S fluid, consisting of small cells, polygonal in outline, with homogeneous cell-substance and round nuclei without nucleoli. He saw no indication of their function, but suggested a possible connection with the nervous apparatus of the gland. SAVIOTTI (3), in the same year, confirmed the observation, describing the cells of the areas in question as being exactly like those composing the ductules. In some cases a continuity with the ductule-epithelium could be made out. This was contradicted by v. EBNER, (4) who, injecting the pancreatic duct, found none of the injection mass in the areas described by LANGERHANS, and could discover in them no trace of lumen. RENAULT (5) described the pancreas as a lympho-glandular organ, the alveolar tissue being intersected by trabeculae of lymphoid tissue. The nodes of this system of trabeculae were the areas described by LANGERHANS, which were called by RENAULT "points folliculaires." KÜHNE and LEA (6), in 1882, described, in the pancreas of the living rabbit, glomeruli with wide tortuous capillaries, which were even more plainly seen in injected specimens. In hardened specimens these glomeruli were found to correspond with areas which they named "intertubular cell-clumps" (*Zellenhäufchen*). Their description of the cells composing them differs in no important respect from that of LANGERHANS and other observers, but they described, in addition a capsule of connective tissue, surrounding each clump and cutting it off completely from the neighbouring alveoli. They found them also in the dog, cat, man, and, particularly large size in the monkey (*Macacus cynomolgus*). They suggested that the clumps were of the nature of small lymph glands. PODWYSOZZKI (7) described them as being very similar in appearance to lymph-follicles, but was of opinion that they were not really lymphatic tissue, and suggested the name "pseudofollicles."

GIBBES (8) found the islets in the dog, cat, guinea-pig and ape, and discussed their nature and function, but without reaching a definite conclusion.*

Up to this point all observers had regarded the islets as permanent structures, quite distinct from the secreting acini. In 1886 LEWASCHEW (9) first pointed out the great variation in their abundance in different specimens of pancreas from dogs and cats. He saw no capsule, but in many cases an apparent continuity between an islet and adjacent alveoli. In many alveoli he found one or more cells resembling those of the islets, and others intermediate between these and normal alveolar cells. All intermediate stages could be found between a group of normal alveoli and a fully formed islet. The islets were more abundant in the pancreas of a fully fed or a pilocarpinised animal, than in that of an animal starved for a few days. His injection results were somewhat uncertain, but in some cases he found lumina, like those of alveoli, in the islets. LEWASCHEW, then, regarded the islets as groups of alveolar cells, altered by fatigue, and supposed that they were reconverted into alveoli during rest.

This view of the islets, as consisting of alveolar cells altered by fatigue, has been held also by DOGIEL (10), PISCHINGER (11) MANKOWSKY (12), TSCHASSOWNIKOW (13) and STATKEWITSCH (14). STATKEWITSCH found that a similar change was caused by prolonged starvation. Of the others, PISCHINGER and MANKOWSKY agree with LEWASCHEW as to the reconstitution of alveoli from the islets during rest; TSCHASSOWNIKOW and DOGIEL regard the islets as consisting of cells in process of degeneration, terminating in absorption.

The embryological investigations of LAGUESSE (15) led him to conclusions of a somewhat similar nature. Already, in 1887, BIZZOZERO and VASALE (16) had pointed out that the islets were abundant in guinea-pig embryos—a fact which, at first sight, seems to tell strongly against LEWASCHEW's view, and has, indeed, been held to do so (OPPEL (17)). LAGUESSE, who first gave to these structures the name of "islets (*îlots*) of Langerhans," described the histogeny of the pancreas in the sheep and the trout. In the sheep embryo the primitive buds, from the anastomosing tubules which form the pancreatic rudiment, are described as being of the nature of islets (*îlots primaires*). These later become converted into secretory acini, which are again transformed into islets. These, after growth by vigorous cell-division, are yet again converted into a larger number of acini. LAGUESSE regards this process, both as a method of growth, and as representing an alternation between externally and internally secreting (exocrine and endocrine) conditions of pancreatic tissue. He considers that the process may continue to some extent throughout life, and thus explains LEWASCHEW's results.

In this connection should be mentioned also the observation of OGATA (18), who,

* The view attributed to him by many German writers, that the islets are embryonic remnants, was not advocated by GIBBES, who, indeed, mentions it, but only to dismiss it as "scarcely probable" in view of their wide distribution.

in the frog's pancreas, discharged by pilocarpine, found patches of tissue in which the cells had quite lost their normal alveolar arrangement, and the similar appearance observed by STEINHARTS (19) and VIR ECKE (20) in Amphibia, and by ELLENBERGER and HOFMEISTER (21) in the horse. None of these observers suggests any connection between such areas and the islets of Langerhans.

It should also be noted that KOLOSSOW (22), who describes bridges of tissue between adjacent gland cells, finds that this connection exists between the cells of the islets and the adjoining secretory cells. He concludes that the islets are composed of glandular elements.

Many observers have maintained, against LEWASCHEW and his supporters, the earlier view which regards the islets as a tissue completely distinct in origin and function from the secreting acini. HARRIS and GOW (23) described the islets in many animals under the name of "secondary cell-groups," and regarded them as a special part of the secretory mechanism, possibly concerned in forming one of the pancreatic enzymes. They found that the cells of the islets became smaller during activity. MOURET (24) and PUGNAT (25) return to the conception of the islets as consisting of lymphoid tissue. JAROTSKY (26) attributes LEWASCHEW's results to the use of a fixative so imperfect as alcohol. Using corrosive sublimate, he never found intermediate forms at any stage of activity. He suggested that the islets furnish a prezymogen, carried in the lymph-stream to the alveoli, and was the first to point out the greater richness in zymogen granules of the alveoli immediately round an islet. This latter was most marked in starving animals and in those fed on fat, least so in those fed on carbohydrate diet. DIAMARE (27) denies the variation of islets with activity, and the presence of intermediate forms. He found, that the small bodies described in the abdominal cavity of fishes by STANNIUS (28) resembled islets in structure. He regards the islets as special epithelial structures, probably furnishing an internal secretion. A similar view is held by MASSARI (29) and by V. EBNER (30). GIANELLI and GIACOMINI (31), in the islets of reptiles, describe an alveolar structure and the presence of ducts. The alveoli of the islets are sometimes continuous with those of the general secreting tissue. They do not, however, adopt LEWASCHEW's theory, but, like HARRIS and GOW, regard the islets as furnishing a special constituent of the secretion. The view of the islets as furnishing the internal secretion of the pancreas has stimulated the investigation of these structures in cases of diabetes mellitus, and a considerable volume of conflicting evidence has been obtained. OPIR (32), who first pointed out the preponderance of islets in the splenic end, as compared with the rest of the pancreas, in mammals, examined the pancreas in a number of cases of diabetes. In a certain number he found the islets affected by hyaline degeneration, or by inflammatory change, which in some cases was selective, leaving the alveoli intact. Similar results were obtained by WEICHELBAUM and STANGL (33), HÄRZOG (34), SROBOLEW (35), and GENTÉS (36), though each found a certain proportion of cases with intact islets. Many other observers have failed

to find evidence connecting diabetes with disease of the islets (KASAHARA (37), WRIGHT and JOSLIN (38), V. HANSEMAUN (39), DIECKHOFF (40), M. B. SCHMIDT (41), GUTMANN (42)). In glycosuria, produced by injection of adrenalin (HERTER (43)) or of leucomaines (LÉPINE (44)), no histological changes in the pancreas were detected.

Of cases of pancreatic disease, without diabetes, the various observers named record a certain number in which the islets were unaffected, but others in which the islets were diseased, scarce, or even indistinguishable.

The evidence as to the relation of the islets to carbohydrate metabolism is, therefore, at best inconclusive.

In the same connection observations have been made, as to the effect, on the structure of the pancreas, of the various procedures by which it has been found possible to destroy its function of external secretion, without affecting the carbohydrate metabolism—ligature of the duct, injecting the duct with oil, or removing the gland from its normal connections and forming a subcutaneous graft. MOURET (45) found that all the pancreatic tissue disappeared, leaving only certain irregular groups of cells, which he does not seem to have regarded as islets. MANKOWSKY (12) found that both the alveoli and the islets disappeared, leaving nothing but ducts and connective tissue. SCHULTZE (46), SSOBOLEW (32), LAGUESSE (47) found that the alveoli rapidly disappear, leaving only ducts and islets embedded in connective tissue. According to LAGUESSE, many islets are new-formed by budding from the ducts. Ultimately these also undergo sclerosis and disappear.

There are thus three principal theories of the nature and functions of the islets of Langerhans, each supported by a certain amount of evidence: (1) That they are the result of a transformation, temporary or permanent, of the ordinary secreting tissue of the pancreas; (2) that they are a special part of the secreting tissue, furnishing a particular constituent of the secretion; (3) that they are entirely independent structures, analogous to such ductless glands as the medulla of the adrenal bodies or the anterior lobe of the hypophysis cerebri, and furnish an internal secretion essential for normal carbohydrate metabolism. The theory put forward by LAGUESSE combines the first and third of these. The evidence here to be presented is entirely in favour of the first. Its relation to the others will be discussed later.

iii. *Histological Methods.*

The animals used were the dog, cat, rabbit and toad. After trying a number of fixatives I found the most generally useful to be a mixture containing corrosive sublimate and formaldehyde. This was made fresh as required by mixing three or four volumes of a saturated watery solution of the sublimate with one volume of strong formal (about 40 per cent.). It, therefore, contained 8–10 per cent. formal-

dehyde. In some cases corrosive sublimate alone was used. The pancreas of dog or cat was cut into thin slices: the rabbit's pancreas, being spread out into a thin layer in the mesentery, needed no such section; the toad's pancreas was hardened entire. The tissue was left in the hardening fluid for 24 hours, washed for 24 hours in running water, passed through alcohols and xylol and embedded in paraffin in the usual way.

In the case of the mammals the sections were cut a thickness varying from 2-4 μ . The toad's pancreas being of looser construction, it was found better to cut it into 5 μ sections for study of the general topography of the gland.

In almost all cases the sections were stained with eosin and toluidene-blue. The stains were used in 1-per-cent watery solutions. The sections, fixed to the slide and freed from paraffin by xylol, were brought through alcohols to dilute tincture of iodine, in which they were left till deeply yellow, to remove excess of corrosive sublimate. The iodine was washed out in 60-per-cent. alcohol, and the sections brought into distilled water. They were then stained with the eosin solution until distinctly overstained, 5-10 minutes, varying with different specimens. The excess of eosin was removed by 60-per-cent. alcohol, until the zymogen granules could be seen under a low power stained very distinctly deeper than the rest of the section. The eosin was then fixed with dilute acetic acid, the sections washed again in distilled water, and stained for $\frac{1}{2}$ -2 minutes, according to the rapidity of staining, with toluidene-blue. After washing in water again, they were passed through alcohols to 95 per cent., in which they were left till sufficient toluidene-blue was removed to give clear differentiation; then through absolute alcohol into xylol; and finally mounted in Canada balsam.

iv. *The Resting Pancreas of the Mammal.*

It should be pointed out, in the first place, that the term "resting" is more convenient than accurate. In a mammal, such as the rabbit, the stomach and small intestine practically always contain food and the pancreas secretes continuously. In the dog the process is more intermittent, but the stomach and small intestine generally contain some remains of food even after 24 hours' fasting. In the cat, under such conditions, the stomach and duodenum are practically empty, and there is a genuine intermission. If it be borne in mind that by "rest" is meant the condition of the gland, taken from an animal unfed for about 24 hours, but otherwise normally nourished, and that the word is thus applied to a condition of activity probably very different in the three animals referred to, it will be convenient to use the term in contrasting such glands with those in which excessive activity has been provoked by artificial means. In a section of such a resting pancreas, stained, as above described with toluidene-blue and eosin, the base of each cell of a secreting alveolus and the chromatin of its nucleus stain deeply with toluidene-blue, the large nucleolus or

nucleoli, and the secretory granules which fill the inner zone of the cell stain bright red with eosin. Among the deeply stained alveoli the ductules and the islets of Langerhans stand out clearly, relatively pale. The epithelium of the ductules consists of pale cubical cells, with oval nuclei containing a faint network which stains purple with both dyes and a small eosinophil nucleolus. This ductule epithelium can frequently be seen continued into the lumen of an alveolus as the centro-acinary cells, which possess nuclei of exactly the same type. The islets of Langerhans consist of irregular polygonal cells which, for the most part, are stained like the ductule epithelium, both as regards cell-substance and nuclei. A few of the nuclei, however, are irregular, shrunken, and deeply stained. Occasionally, but rarely in the resting gland, an islet can be found which is apparently continuous with the epithelium of a ductule, and the similarity of the islet-cells to those of the epithelium is then very obvious.

The abundance and size of the islets varies in different glands, and in different parts of the same gland. As ORIE pointed out, the islets are always more abundant and larger at the splenic end than in the rest of the gland.

By comparison of a number of sections from different specimens of the three species examined, the sections being taken always from the splenic end of the pancreas, it is seen that the islets are most abundant in the pancreas of the rabbit, less so in that of the dog, and still less so in the cat; and it is worth noting that the same order applies to the relative continuity of the digestive process in these three animals.

It is possible, also, in the pancreas of the dog or cat, roughly to predict the abundance or scarcity of islets by the naked-eye appearance of the fresh gland. It will be found that a cat's or dog's pancreas, for example, which has the opaque white appearance of a fully loaded gland has few islets even at the splenic end, while one which, from some unknown difference in the process of nutrition, has the translucent grey appearance of the discharged, or partly discharged, gland will show a relatively greater abundance of islets. Fig. 1, Plate 2, shows part of a section across the splenic end of a dog's pancreas, which has about the average abundance of islets in that animal. Fig. 2, from a cat, is from a pancreas which contained an unusually large number of big islets, which, even at the splenic end, are usually rare in the cat.

The structure of the islets, as seen under a high power, is different in the rabbit from that seen in the cat and dog. The islets in the rabbit stand out less clearly from the surrounding alveolar tissue than those of the cat and dog. This is due to the fact that the islet-cells in the rabbit stain fairly well with toluidene-blue, and better with eosin. The result is a pinkish-purple tint of all the islet-cells. The same is true of the ductule epithelium of the rabbit. There is no real difficulty, however, under low or high power, in recognising the islets. They are seen to be usually of rounded, compact form, and, at first sight, sharply marked off from the surrounding secretory alveoli. With a high power many of the islets show a division of the cells, by intervening connective tissue and blood capillaries, into packets which

have a shape strikingly similar to that of the secretory alveoli. In such, an outer layer of larger cells can be distinguished from an inner layer of smaller cells, exactly like centro-acinary cells. The appearance, in fact, is exactly that of a group of alveoli in which the secreting cells have lost their characteristic basal basophil staining and zymogen granules, their nuclei having become more centrally placed, and assimilated to those of the centro-acinary cells, and in which the lumen has become obliterated by a falling together of the cells. In most of such islets it can also be seen that the sharp circumscription is only apparent, and is not due to a capsule, as described by KUHNE and LEA. The divisions of the islet, of suggestive alveolar shape as described above, can often be found actually continuous, beyond the islet margin, with undoubted normal secreting alveoli, containing zymogen granules, and staining characteristically both in cell-substance and nucleus.

In many islets the appearance of alveolar structure and of continuity with the secretory tissue is not seen. In such islets the cell-substance is reduced, and the cells displaced, so that the nuclei are irregularly crowded together. The blood capillaries are also enlarged and tortuous, so that the islet has the form of a coarse network.

In the dog and cat the islets also vary not only in size and number, but in structure. In cases where the islets are large and abundant—such are commoner in the dog—it is easy to find islets showing traces of alveolar arrangement, some of the cells staining in parts like secretory cells, whilst continuity with the secretory alveoli surrounding the islet is often very clearly seen. Such an islet is shown in fig. 3. In this, a common type of such continuity is seen, the islet tissue being continued into the centro-acinary cells of an adjacent alveolus, of which the secreting epithelium seems, therefore, to form a cap over a prolongation of the islet. In the islet itself traces of alveolar structure are obvious even in the photograph, and in one place cells are seen which, though evidently part of the islet, stain like secretory cells, and contain zymogen granules. On the other hand, sections of islets are found in the cat and dog which have no apparent connection with surrounding alveoli, but consist of closely packed, polygonal cells, filling the meshes of a plexus of large blood capillaries. Between such a structure, however, and that described above, and illustrated in fig. 3, there can be found all conceivable intermediate stages.

About the continuity and connection between secretory and islet tissue there can, I think, be no doubt. The question remains, however, whether such a structure as that shown in fig. 3 represents a new formation of secreting tissue from an islet, or of an islet from secreting tissue. The impression gathered from a consideration of such a preparation by itself is strongly in favour of the latter view. The unequal distribution of the staining reaction, so that an isolated cell, or part of a cell, shows the basophile reaction, which fades away towards its edge; the presence of cells which have no basophile properties, but retain a few eosinophile granules, which appear to be undergoing solution; the faint trace of alveolar outline, like the shadow of a

structure which is being lost, none of these would be expected in a new formation of alveoli. In the same direction points the fact that the abundance of these intermediate forms is greatly increased by such influences as cause increase in the number and size of the islets. To the consideration of these we must now proceed.

v. *The Effect of Secretory Activity on the Mammalian Pancreas.*

For the study of this effect only the cat and dog were taken as types of mammalia. The effect on the rabbit was not observed, since, as shown by BAYLISS and STARLING, secretin makes only a small difference in the rate of its pancreatic secretion.

The dogs and cats were anaesthetised with morphia and A.C.E. mixture, a cannula tied in the pancreatic duct, so that the rate of secretion could be watched, and secretin solution injected into the jugular vein in doses of 5-10 c.c. at such intervals as to keep the gland constantly secreting for many hours, until it began to show signs of exhaustion. In many cases this condition was never attained. In one case, after 14 hours continuous secretion the gland responded to secretin as well as ever, and had the opaque naked-eye appearance of a loaded gland, from which, on section, it showed no recognisable histological difference.

The effect of different specimens of secretin, and the response to the same specimen of different animals, showed unaccountable variations. With a powerful secretin and a responsive pancreas, especially if the animal be bled towards the end of the experiment with each administration of secretin, it is often possible to reduce the gland to such a state of exhaustion, that further doses of secretin produce hardly any, or no secretion.

In making comparisons of exhausted with resting glands, in respect of the abundance of islets which they contain, certain obvious sources of error must be eliminated. It is clearly useless, for reasons stated above, to compare the splenic end of one with the free end of the other gland. One cannot, therefore, remove part of the gland at the beginning of the experiment and compare it with the rest of the gland subjected to secretin. On the other hand, by taking sections from the splenic end of the pancreas from two different animals, even when kept under conditions apparently identical, it is impossible to be certain of eliminating the differences due to individual variation. No difference, therefore, which can come within those limits of variation can be considered conclusive.

I have in all cases taken sections from the splenic end of the pancreas. By comparison of a number of specimens from resting glands with a number of specimens from glands in which activity has been provoked short of exhaustion, whether by pilocarpine or by secretin, one received a general impression of greater abundance of islets in the active glands.

Such are the differences, presumably, observed by LEWASCHEW and his followers, and denied by their opponents. This contradiction of evidence is not difficult to

understand. I have never found, under such conditions, such an abundance of islets as could not be matched by isolated specimens obtained from a resting pancreas. To carry conviction on the point, it would be necessary to cut serial sections through the pancreas, and count and measure the islets.

The case is different when the gland is thoroughly exhausted. The sections from the exhausted pancreas of a dog, shown in figs. 4 and 5, show islets in such abundance and of such a size as are never seen in any part of a resting gland.

All of these islets, moreover, show signs of active formation. The traces of alveolar structure in most of them are very distinct; their outline is irregular, on account of encroachment on the surrounding alveoli; and transitional forms are abundant, both on the margin and in the interior of the islets. In fig. 5 is seen practically the whole of a lobule which, for the most part, is converted into tissue indistinguishable from that of the islets, but still contains many incompletely altered alveoli which are visible in the photograph.

Comparing the cat's pancreas in fig. 6 with that in fig. 2, the contrast is not so striking. Fig. 2, however, shows an abundance of islets unusually large in the resting cat's pancreas, while the gland shown in fig. 6 was not reduced to complete exhaustion—a condition which I have never yet produced in the cat. It may still be claimed with confidence that fig. 6 shows such an abundance and progressive formation of islets as is never seen in the resting pancreas of the cat.

When the specimens are examined under high powers of the microscope the difference caused is even more evident than with the low magnification shown. It can then be seen that, apart from the large areas of definite islet tissue such as the low power shows, a large proportion of the remaining alveoli show partial change into what must now be called the islet condition, some of their cells having lost their normal staining properties and having become assimilated to the centro-acinary cells.

Another feature, which is, unfortunately, not shown in the figure, is the frequent apparent continuity of the islets with the epithelium of the smaller ductules. The origin of such an appearance is quite clear, if we imagine the area of gland adjacent to and drained by such a ductule undergoing this change, which we have seen to be of such a nature as to assimilate the secretory cells to the centro-acinary cells and the ductule epithelium.

Such preparations afford clear evidence that the conversion of secreting tissue of the pancreas into islets of Langerhans, of which we saw indications in the resting gland, is greatly accelerated and rendered much more extensive by excessive secretory activity.

vi. *Effect of prolonged fasting on the Mammalian Pancreas.*

It might, at first sight, be expected that fasting would have an effect opposite to that produced by secretory activity, and that the pancreas of a starved animal would

contain even fewer islets than the resting pancreas of a normally fed animal. The statement of STATKEWITSCH that starvation caused formation of islets from alveoli, was contradicted by JAROTSKY. The objections to making the matter the subject of deliberate experiment are obvious. An opportunity was afforded by the capture of a stray cat which, from its emaciated condition, had probably been without food for some time. The cat was killed, and the stomach and small intestine found to be empty, but fecal matter was found in the cæcum and colon. The pancreas was small, greyish and translucent, having the appearance of an exhausted gland. Sections from the splenic end showed a pancreas of the discharged type, though a few zymogen granules were present. There was a great abundance of large islets, with clear evidence of progressive formation, as in the gland exhausted by secretin. Such a section, under a low power, is shown in fig. 7 (Plate 3). The abundance of islets is again such as is never seen in a resting pancreas of the cat.

The examination of this one specimen entirely corroborates the statement of STATKEWITSCH. The discussion of the meaning of this change, and of the difficulties involved in this conception of the origin of the islets, must be postponed till the effect of these same conditions on the Amphibian pancreas has been described.

vii. *The Resting Pancreas of the Toad.*

Previous observations on the islets of Langerhans in the Amphibian pancreas have not taken account of the effect of secretory activity. LEWASCHEW could not discover islets in cold-blooded animals, while v. EBNER and DIAMARE, who described them in the frog and in various Amphibia, regarded them as completely independent organs, altogether unaffected by the secretory activity of the rest of the gland. It seemed especially important, therefore, to extend to the Amphibian pancreas these observations of the effect of secretin and of fasting.

The toad was found to bear much better than the frog the effects of long captivity and of the injection of secretin. It was therefore taken as the Amphibian type.

The question first arises as to what can be considered as the resting condition of the toad's pancreas. The animal feeds seldom: in winter not at all. It may be kept alive for a very long time without food; yet it was found that, after a certain period of fasting the pancreas of the toad, like that of the mammal, exhibited changes which cannot be considered characteristic of rest. It is not possible in such an animal to draw a definite line between the state of rest and that of starvation, but it was found that the pancreas taken a month after capture in summer, or three months in winter showed no starvation changes.

The histological method was the same as in the case of the mammalian pancreas, except that the gland was hardened entire and a series of sections cut through the whole. There was thus no danger, in this case, of error due to local variations.

The pancreas of the toad, hardened and stained as described, shows on section an appearance very similar to that of the mammal, as far as the purposes of this investigation are concerned. The most evident differences, under lower power, are the number and prominence of the ducts, the general looseness of structure. The large number of the islets of Langerhans, and the large relative size of some of them. The epithelium of the smaller ducts consists, as in the mammal, of pale cubical cells, with oval nuclei, containing small nucleoli and a scanty chromatin network. The epithelium of the ductules is very obviously continued by the centro-acinary cells, which are, in parts of the gland, so prominent, that many alveoli present the appearance of a number of secretory cells somewhat loosely arranged round a ductule. The islets of Langerhans are abundant, and vary much in size. Some of the smaller islets have the appearance of buds from the smaller ducts, being simply clumps of cells resembling those of the duct epithelium. Some of these buds show an arrangement into a double row of cells surrounding a lumen. In the immediate neighbourhood of such structures may often be found alveoli which differ from them only in the nature of the outer layer of cells, which in this case is formed of typical secretory cells, with basophil outer zone, inner zone filled with eosinophil granules, and a nucleus containing a large nucleolus and a network with large masses of chromatin. A few of the cells of the outer layer, however, have nuclei like those of the inner layer—the centro-acinary cells in this case—and their cell-substance shows only faint basophil staining and few zymogen granules. Though the transition between these two forms is sufficiently clear, it is difficult to be certain as to its direction—whether we have here a new bud from a ductule forming an alveolus, or an alveolus undergoing a change analogous to that seen in the mammalian islets, and here giving rise to the appearance of a bud from a ductule. The absence of signs of nuclear multiplication, and the presence of zymogen granules in cells with faint basophil staining and nuclei of the centro-acinary type, seem to point to the latter of the two possibilities. This view is confirmed by study of the large compound islets, consisting of several divisions, separated by septa containing blood capillaries. In such an islet one division has frequently the structure of one of the simple islets just described, and is continuous with yet unaltered or partially altered alveolar tissue. The rest of the islet usually has the structure which must be considered as typical of the islets of the resting toad's pancreas, and which is illustrated in fig. 8. Such a typical islet appears quite shut off and separate from the surrounding secretory alveoli. It consists of a number of columns or compartments of cells which stain similarly to the ductule epithelium, but are mostly elongated in form and have a palisade arrangement. The nucleus of such a cell is sometimes correspondingly elongated, having a nucleolus at each end; in other cases the cell contains two rounded nuclei, indicating that the elongated form probably represents a stage of amitotic division. A compartment of such an islet can often be found in which the cells show an alveolar arrangement into two layers round an indistinct lumen.

In such a case the cells of the outer layer have spherical nuclei, which are of the type found in secretory cells, while the cell-substance shows basophil staining, and, in a few cases, a small number of eosinophil granules. Such an appearance, I believe, represents the early stage of reconstruction of an alveolus from the islet. Fully formed and loaded alveoli are often seen, which yet appear to be included in the islet outline. An instance of this is to be seen near the upper border of the specimen in fig. 9. In spite of much searching, however, I have not been able to trace a series of intermediate stages in the formation of such an alveolus.

Two other points in the histology of the resting pancreas of the toad are deserving of mention and further investigation. The first, which has some relation to the appearance just described, is the fact that the alveoli immediately surrounding such a "resting" islet are more fully loaded with secretory granules than those in the rest of the gland. This was noticed by JAROTSKY in the mammal, and by him attributed to the formation of a prezymogen by the islets. I have also noticed it in the resting dog's pancreas, but the difference in the toad is far more striking. These fully loaded alveoli are not always seen surrounding the islet on every side. At one point the islet may be growing, by the breaking down into islet tissue of an imperfectly loaded alveolus, while on its other sides it may be surrounded by the fully loaded alveoli, which, by their concentric disposition round the islet, give the impression of having formerly formed part of it. Another process, of which the significance is not quite clear, is generally to be seen at some point of the resting pancreas of the toad. This is a vigorous nuclear multiplication, giving, under a low power, the appearance of a localised infiltration with round cells. When seen under a high power, this is seen to be due to mitotic nuclear division, the nuclei implicated being chiefly those of the alveoli and ductules, but, to some extent, apparently those of the connective tissue also. In the pancreas of the winter toad, the process was widespread, mitotic division of nuclei being found chiefly in the neighbourhood of the ducts. A patch of such nuclear multiplication, which was found, with a high power, to contain many mitotic figures, is seen near the division between the two lobes in fig. 9. Some of the cell-bodies containing these nuclei retain traces of their secreting characters, but they are in most cases lost. We have here, then, another method by which the alveolar tissue can become converted into areas filled with cells of homogeneous type.

The whole process is by no means clearly made out as yet, but enough has been seen to make it clear that there is a constant tendency, on the part of the secretory tissue of the toad's pancreas, to revert to what we may consider a more embryonic type. This must obviously be balanced by a constant reconstruction of secretory tissue, and of this process we have seen some indication. We have yet to consider the effect of the conditions which, in the case of the mammalian pancreas, were found to cause extensive change of the secretory tissue.

viii. *Effect of Prolonged Fasting on the Toad's Pancreas.*

For reasons given above, it is necessary to keep a toad for a prolonged period without food if we wish to observe the effects of inanition. Toads were taken, on two different occasions, which had been in the laboratory for 6 months and 5 months respectively. In both the changes found were of the kind expected—a widespread conversion of alveoli into tissue resembling the islets of Langerhans. In most cases this amounted merely to a wider distribution, affecting in certain parts of the gland nearly all the alveoli, of the process, described in the resting gland, of direct conversion of alveolar into islet tissue. The pancreas, of which a section is reproduced in fig. 10, was taken from a toad which had been in the laboratory from the beginning of October to the end of March. This showed a more extensive change, and contained large areas in which only traces of alveoli could be detected. In some of these areas a few mitotic figures were found. These areas had the structure of the newly formed portions of islets seen in the resting gland. No indications of the reconstruction of alveoli were found in this specimen.

If we adopt the idea of a balance of two opposing processes in the normal resting gland, we may explain this result of fasting as due, either to an acceleration of the conversion of alveoli into islet tissue, or to an inhibition of the reconstruction of alveoli from islets, or to a combination of the two.

ix. *Effect of Secretory Activity on the Toad's Pancreas.*

Secretion of the pancreas was brought about by injection of ordinary secretin solution, prepared from the dog's duodenum, into the dorsal lymph-sac. No measure of the rate of secretion was possible, but it was observed, in a toad which had received such an injection and was pithed during its absorption, that fluid could be detected, with a hand-lens, exuding from the papilla of the pancreatic duct into the duodenum. For the rest, the evidence of secretory activity was purely histological, and it was taken for granted, that the discharge of all or most of the large and easily preserved zymogen granules was sufficient indication of prolonged and vigorous activity. Such a change was produced by the injection of secretin into the dorsal lymph-sac. The secretin was given in doses varying with the size of the toad, each injection being sufficient to fill the lymph-sac. The process was repeated as often as the fluid was found to be absorbed, and was continued for 2, 3 or 4 days. In this way a large toad sometimes received as much as 40 cub. centims. of secretin solution. If the toad was killed shortly after the last injection, it was found that the ducts and tissue spaces of the pancreas were distended with fluid. The animal was, therefore, left for some hours after the last dose of secretin had been completely absorbed. In a few instances the toad received its final injection on

the evening of one day and was found dead on the morning of the next. In such cases the pancreas was only preserved, when it was clear that the animal was only recently dead, *rigor mortis* not having set in, and the muscles still responding to stimulation of the nerves. The facts that in such a case a small piece of liver gave a normal histological picture, and that the changes in the pancreas were precisely similar to those found in other specimens from toads similarly treated, but killed by pithing immediately before preparation, should suffice to exclude the suspicion of *post-mortem* change.

Eleven toads were thus injected, and the effect was of the same kind in all cases, though it varied somewhat in degree. This might be expected from the varying effect of different preparations of secretin and the varying response of different animals as seen in experiments on mammals. There was a very great increase of the tissue, which we have now frequently described, and have called islet tissue. This increase is due, as always, to a conversion of the secretory alveoli. In the specimen, of which a section is reproduced in fig. 11, the change is very extensive. The exhaustion is very complete, no zymogen granules being found in any part of the section, and a very large proportion of the whole tissue of the gland has undergone the change into islet tissue. A few alveoli retain their identity, and a large number can be seen in various stages of conversion. The condition of the whole gland, in fact, is very similar to that of the small portion of dog's pancreas shown in fig. 5. Scattered in this transitional tissue are several fully formed islets of the resting type, which were presumably present before the injection of secretin. This specimen was obtained from a winter toad. A control toad, not injected with secretin, gave pancreas sections very similar to that shown in fig. 9.

Another feature of this exhausted gland, not included in fig. 11, was a very large area studded with nuclei, which nearly all showed mitotic figures. The production of these mitotic figures is thus also clearly accelerated by the action of secretin. In fig. 12 is seen, under higher magnification, a portion from another pancreas less completely exhausted, the discharge of zymogen granules being not quite complete. In the middle of the field is seen a large area composed of faintly staining cells, with nuclei so faintly stained as to be visible with difficulty in the photograph. A few of the cells can be seen, in the original section, to retain traces of basophil staining. They show no definite arrangement, either into the alveoli of secretory tissue, or the columns of the "resting" islet. A clump of cells, seen in the middle of this area, have lost their alveolar arrangement, but retain their staining properties and a few zymogen granules. Many cells of surrounding alveoli have become assimilated to the cells of this forming islet, which has thus the appearance of eating its way into the adjacent tissue. In one corner a longitudinally cut lumen is seen, but whether it is that of a pre-existent ductule, or has arisen *de novo* in the islet-area, it is impossible to say. The cells are exactly similar to those of ductules elsewhere in the section.

The whole section contains great numbers of such areas, which, indeed, are almost continuous, very few unaffected alveoli being found.

The specificity of these effects was proved by the injection, in precisely similar manner and quantities, of an extract prepared from the lower end of the ileum. This has been shown to contain the depressor substance present in secretin solutions, but to be without stimulating effect on the pancreas (BAYLISS and STARLING). Other toads were injected with salt solution. In neither case was any perceptible effect produced.

Infusion for several hours of the whole toad with secretin, through a cannula tied into the bulbus arteriosus, caused some change of the same kind as that described, but far less in extent. The cells were probably killed quickly. Ileum extract and salt solution, similarly perfused, had no appreciable effect.

x. *Effect of Occluding the Pancreatic Duct in the Mammal.*

The statement of several observers, that occlusion of the pancreatic duct is followed by a degeneration and disappearance of alveolar tissue, while the islets remain intact, is often put forward in evidence of the independent origin and function of the islets. Though it is evident, that the disappearance of lumina and rearrangement of cells, which has here been described, would sufficiently account for the supposed immunity of the islets from the effects of duct-ligature, it seemed advisable to repeat the observations.

In the pancreas of the dog there are two ducts, of which only one is easily reached for the tying operation. The shortness of the ducts also makes it difficult to avoid the re-formation of a lumen by a process of ulceration, if the ducts are simply tied. The method used by SCHIFF, HEDON, and others, of injecting paraffin into the lower duct was, therefore, adopted. The operation was kindly performed for me by Professor STARLING. The dog was anaesthetised with morphia and A.C.E. mixture. The paraffin injected had a melting point of 40° C. The aseptic precautions were perfectly successful, and the dog was kept for 4 weeks after the operation. Only one such experiment was made.

In the rabbit there is only a single duct. It is quite easy to doubly ligature this, and remove about $\frac{1}{4}$ inch of duct between the ligatures, thus minimising the risk of restoration of the channel. I performed this operation on three rabbits. These were anaesthetised with morphia and ether. They bore the operation well, the wounds healed without suppuration, and the rabbits were killed 3, 7 and 15 days respectively after ligature of the duct.

In the dog a cast of paraffin was found blocking the duct up to the splenic end of the pancreas. The gland looked shrunken and irregular. Microscopically this was found to be due to an interstitial fibrosis. The connective tissue was everywhere

much increased. Many lobules of practically normal alveoli containing normal islets could be found. Other lobules, however, were reduced to small central cores of pancreatic tissue, surrounded by dense layers of connective tissue. These pancreatic remnants were in some cases still obviously composed of secretory alveoli, but of alveoli which were undergoing a change very similar to that now familiar in the formation of islets. In neighbouring lobules the change had proceeded further and the remnant was very similar in appearance to islet tissue. There was no indication that pre-existent, fully formed islets had escaped destruction, but it was clear that the whole pancreas was undergoing a change which would ultimately reduce it to a mass of connective tissue, including remnants of pancreatic alveolar tissue, which, as in all circumstances of defective nutrition, would assume a form resembling that seen in the islets of the normal gland. This I believe to be the true explanation of the supposed immunity of the islets from the destructive process consequent upon occlusion of the duct.

In the rabbit the effect was somewhat different. After 3 days' ligature there was distension of the ducts and indistinctness of the alveolar cells. The alveolar cells had, to a large extent, lost their characteristic staining properties and their secretory granules. It was difficult to distinguish the islets of Langerhans; areas showing the characteristic islet arrangement and staining of cells were apparently quite continuous with the degenerating alveoli surrounding them.

After 7 days, very many of the alveoli were greatly distended, and their secreting cells flattened, so that the section appeared thickly studded with sections of ducts. Between these were other alveoli in which the cells were becoming disordered, losing their characteristic staining properties, and becoming assimilated to the islets. It was impossible to draw any sharp line of distinction between islets and alveoli undergoing these changes, or to determine whether the islets present in the pancreas before the operation had survived. There was a slight degree of interstitial fibrosis.

After 15 days the fibrosis had proceeded much further. A large part of the pancreas was replaced by fibrous tissue. In this were embedded the remains of pancreatic lobules. In these could now be seen no traces of normal alveolar structure. Many of the alveoli by distension of the lumen and alteration of the epithelium has been converted into structures resembling branching and tortuous ductules, of which the lumina were not so large as those seen 7 days after ligature. In some cases continuous with these, in others quite separate and surrounded by a capsule formed of the invading fibrous tissue, were large areas having the structure of islets of Langerhans. They were more numerous and much larger than the islets seen in the normal rabbit's pancreas, but in no case showed an alveolar structure. All showed the radial arrangement of cells to blood capillaries, which was noticed in the fully formed islet of the rabbit's pancreas. Though the shrinkage, due to fibrous contraction, might account for the great number of these structures, on the assumption that they were pre-existing islets which had survived the

destructive process, such a theory will not account for their large size. A comparison with the pancreas 7 days after ligature, in which islets were with difficulty distinguished at all, renders inevitable the conclusion that these islets are, at least in part, formed from portions of the alveolar tissue of the pancreas, in the course of the changes following ligature of the duct.

In no case was I able to observe the proliferation of duct epithelium, with nuclear mitosis, described by SSOBOLEW and LAGUESSE, and connected by the latter with a formation of new islets.

It is of interest to note that a formation of islets from alveolar tissue, similar to that which I have described, was observed by M. B. SCHMIDT in a case of obstruction of the human pancreatic duct by a calculus. In this case the obstruction was associated with severe diabetes. He observed similar changes as the result of senile atrophy.

xi. Summary and Discussion.

The results of this investigation may be summarised as follows :—

1. The islets of Langerhans are not independent structures of separate origin to the rest of the pancreas, but are formed by certain definite changes in the arrangement and properties of the cells of the ordinary secreting tissue. The changes are of such a kind as to assimilate all the cells to those forming the epithelium of the ductules and the centro-acinary cells, thus bringing about a reversion to embryonic type. The lumina disappear in this process, and all the cells are brought into more intimate relation with the blood capillaries. Such changes have been observed both in mammals and Amphibia.

2. In the pancreas of the toad some evidence was found of cell-multiplication in the islets, and of reconstruction of alveoli from them. Such evidence is at present wanting in the case of mammals.

3. The change from the secreting to the "islet" condition is greatly accelerated, both in mammals and Amphibia, by exhaustion of the gland by means of secretin. True exhaustion of the mammalian gland was not found possible unless the animal was also bled. This suggests that secretin stimulates both anabolic and katabolic activity of the pancreatic cells, and that anabolism must be otherwise depressed if the exhaustion effect is to be produced.

4. The proportion of islet tissue to secreting tissue is also increased by prolonged fasting. In other words, disappearance of the stored material of the secretory cells, whether by discharge into the duct, to produce the secretion, or by absorption into the blood and lymph, when the nutrition of the body fails, is attended by increased formation of islets from secretory alveoli.

5. Occlusion of the duct causes a disappearance of most of the pancreatic tissue in the course of a few weeks. That which escapes destruction assumes a form

resembling the islets, but the already existing islets exhibit no special immunity from the destructive effects of the operation.

Several points still require elucidation. In the first place, the evidence of reconstruction of alveoli from islets is as yet inadequate.

It is quite evident that, if islets are in constant process of formation from alveoli, there must be a constant disappearance of islets and new formation of alveoli to maintain the balance between the tissues. In the toad there is evidence of reconversion of islets into alveoli, and though there is no direct evidence of such a process in the mammal, it seems unlikely that the course of events should differ fundamentally from that in the toad.

The scattered and localised nature of the process of islet formation presents no real difficulty of explanation. It is quite in accordance, indeed, with the markedly different conditions of activity, apart from islet formation, which can be seen in different lobules, and in different and even neighbouring alveoli of the same lobule. In the normal pancreas the whole gland does not secrete simultaneously with equal vigour, or at least does not equally maintain, in all its alveoli, the balance between output and new formation of secretory granules. We have seen that by artificially producing a simultaneous exhaustion of the whole gland or a whole lobule, we get no longer a localised process of islet formation, but an approach to a conversion *en masse*.

The difficulty of the rich blood supply of the islets has already been discussed by PISCHINGER, who attributed it to a weakened resistance to injection—a lowering of capillary pressure, caused by shrinkage of the cells forming the islet. It is not necessary, however, to suppose that the change is merely one of this kind. The observations of the rich blood supply of the islets have been made on the full-formed islets of the resting gland. An islet like that shown in fig. 12, in the earliest stage of its formation, has a blood supply differing in no respect from that of the rest of the gland. Whether the development of the blood supply in such an islet as that in fig. 8 is due merely to widening of capillaries with the rearrangement and decrease in volume of the cells, or to new formation of capillaries, I have not been able to determine. The toads in which I hoped to study these changes, by keeping for some time after treatment with secretin, did not survive. No *a priori* impossibility, however, can be urged against either process.

In regard to the supposed internal secretion of the islets, these observations furnish no evidence in either direction. It may be, as LAGUESSE has suggested, that the passage from alveolar to islet form denotes a change of polarity in the cells, which, having previously secreted into the lumen, now secrete into the blood vessels. I have never been able to discover the "endocrine" granules, which he and others have described, and it might be suggested with equal plausibility that the process of *formation* of the islets, involving the absorption of zymogen granules, nuclear chromatin and other cell-constituents, is the essential factor in the internal secretion of the pancreas. Be that as it may, the one function of the islets which is indicated

by these actual observations is that of pancreatic growth, the islet stage in the toad, at any rate, being that in which cell-multiplication takes place—an observation which tallies with the embryological account given by LAGUESSE.

The pathological observations, on the selective action of certain forms of inflammation and degeneration, need not indicate more than that pancreatic tissue in the islet stage shows weakened resistance to certain morbid processes.

The evidence as to the relation of such changes to diabetes is at present too inconsistent to do more than furnish support to preconceived theories of the function of the islets.

In conclusion I wish to express my gratitude to Professor STARLING for kind permission to work in his laboratory. I am deeply indebted to him, and also to Dr. BAYLISS, for constant advice and personal assistance, and for the supply of much valuable material.

FIGURES.

Where not otherwise stated the preparations were hardened in the corrosive sublimate and formaldehyde mixture, and stained with eosin and toluidene-blue.

PLATE 2.

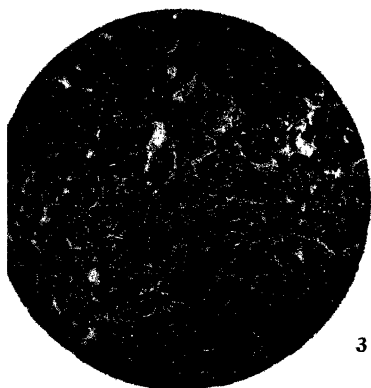
- Fig. 1. Pancreas of dog, splenic end, resting. $\times 18$.
- Fig. 2. Pancreas of cat, splenic end, resting, corrosive. $\times 18$.
- Fig. 3. Islet of Langerhans, pancreas of dog, resting. $\times 225$.
- Fig. 4. Pancreas of dog, exhausted by secretin, splenic end. $\times 18$.
- Fig. 5. Pancreas of dog, exhausted by secretin, splenic end. $\times 36$.
- Fig. 6. Pancreas of cat, nearly exhausted by secretin, splenic end. $\times 18$.

PLATE 3.

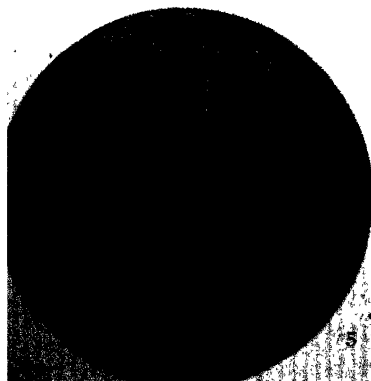
- Fig. 7. Pancreas of cat, starved. $\times 16$.
- Fig. 8. Islet of Langerhans, resting pancreas of toad. $\times 225$.
- Fig. 9. Pancreas of toad, resting. $\times 45$.
- Fig. 10. Pancreas of toad, starved. $\times 45$.
- Fig. 11. Pancreas of toad, exhausted by secretin. $\times 40$.
- Fig. 12. Islet of Langerhans, active formation, pancreas of toad exhausted by secretin. $\times 250$.



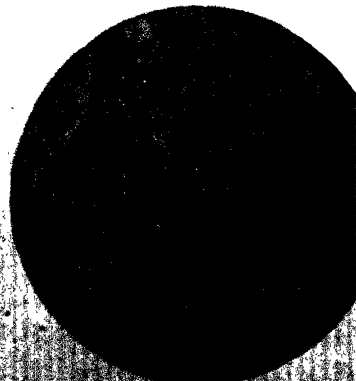
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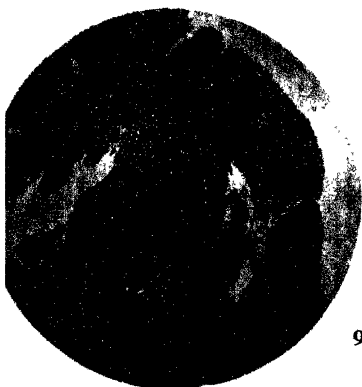
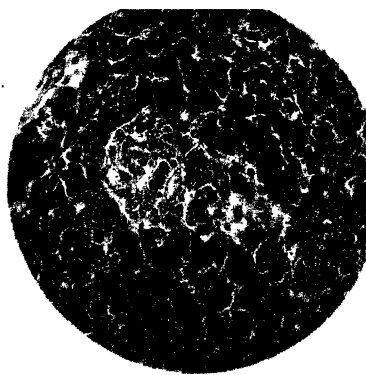


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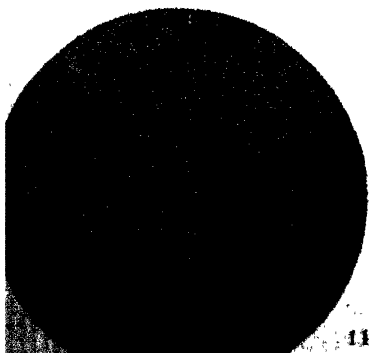
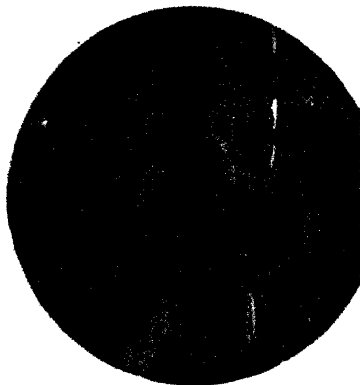




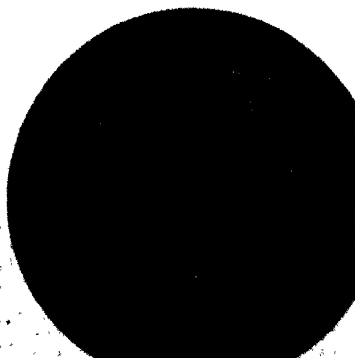
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BIBLIOGRAPHY.

1. BAYLISS and STARLING. 'Journal of Physiology,' vol. 28, p. 325, 1902; vol. 29, p. 174, 1903.
2. LANGERHANS. "Beiträge z. Mikr. Anat. d. Bauchspeicheldrüse," 'Inaug. Diss.' Berlin, 1869.
3. SAVIOTTI. 'Arch. f. Mikr. Anat.,' vol. 5, p. 204, 1869.
4. V. EBNER. 'Arch. f. Mikr. Anat.,' vol. 8, p. 481, 1872.
5. RENAULT. 'Comptes Rendus,' vol. 89, p. 247, 1879.
6. KÜHNE and LEA. 'Untersuch. aus d. Physiol. Institut, Heidelberg,' vol. 2, p. 448, 1882.
7. PODWYSOZZKI. 'Arch. f. Mikr. Anat.,' vol. 21, p. 765, 1882.
8. GIBBES. 'Quart. Journ. Micros. Science,' vol. 24, p. 183, 1884.
9. LEWASCHEW. 'Arch. f. Mikr. Anat.,' vol. 26, p. 453, 1886.
10. DOGIEL. 'Arch. f. Anat. u. Phys. (Anat. Abth.),' 1893, p. 117.
11. PISCHINGER. "Beitr. z. Kenntniss d. Pankreas," 'Inaug. Diss.' Munich, 1895.
12. MANKOWSKY. 'Arch. f. Mikr. Anat.,' vol. 59, p. 286, 1902.
13. TSCHASSOWNIKOW. 'Ueber d. Struktur u. function. Veränder. d. Pankreaszellen.' Warsaw, 1900. (Ref. Mankowsky. Original in Russian.)
14. STATKEWITSCH. 'Arch. f. experim. Pathologie,' vol. 33, p. 453, 1893.
15. LAGUESSE. 'C. R. Soc. de Biol.,' ser. 9, vol. 5, p. 819, 1893; ser. 10, vol. 2, p. 699, 1895.
 ——— 'Journ. de l'Anat. et de la Physiol.,' vol. 31, p. 475, 1895; vol. 32, pp. 171 and 209, 1896.
 ——— "Verhandl. d. Anat. Gesellsch.," 'Anat. Anzeiger,' vol. 13 (Ergänzungsheft), p. 43, 1897.
- BIZZOZERO and VASALE. 'Virchow's Archiv,' vol. 110, p. 155, 1887.
- OPPEL. 'Vergleich. Mikr. Anat.,' vol. 3, p. 805. Jena, 1900.
- OGATA. 'Arch. f. Anat. u. Phys. (Phys. Abth.),' 1883, p. 405.
- STEINHAUS. 'Beiträge z. Path. Anat. u. allgem. Path.,' vol. 7, p. 367, 1890.
- VER ECKE. 'Arch. de Biol.,' vol. 13, p. 61, 1893.
- ELLENBERGER u. HOFMEISTER. 'Arch. f. wiss. u. prakt. Thierheilk.,' vol. 11, p. 141, 1885.
22. KOLOSSOW. 'Arch. f. Mikr. Anat.,' vol. 52, p. 1, 1898.
23. HARRIS and GOW. 'Journal of Physiology,' vol. 15, p. 349, 1894.
24. MOURET. 'C. R. Soc. de Biol.,' ser. 10, vol. 1, p. 731, 1894.
25. PUGNAT. 'Journ. de l'Anat. et de la Physiol.,' vol. 33, p. 267, 1897.
26. JAROTZKY. 'Virchow's Archiv,' vol. 156, p. 409, 1899.

27. DIAMARE. 'Internat. Monatschr. f. Anat. u. Phys.,' vol. 16, pp. 155 and 177, 1899.
28. STANNIUS. 'Lehrb. d. Vergl. Anat.' (STANNIUS and SIEBOLD). Part 2. Berlin, 1846.
29. MASSARI. 'Rend. R. Accad. dei Lincei.' Rome, vol. 7, p. 134, 1898.
30. v. EBNER. 'Kölliker's Handbuch d. Gewebelehre,' vol. 3, 1st half, p. 255. Leipzig, 1889.
31. GIANELLI and GIACOMINI. 'Proc. verb. d. R. Accad. d. Fisiocrit.' Siena, 1896.
32. OPIE. 'Bull. Johns Hopkins Hospital,' vol. 11, p. 205, 1900.
——— 'Journ. of Experim. Medicine.' New York, 1901, pp. 397 and 527.
33. WEICHELBAUM and STANGL. 'Wiener Klin. Wochenschr.,' vol. 14, p. 968; vol. 15, p. 969.
34. HERZOG. 'Virchow's Archiv,' vol. 168, p. 83, 1902.
35. SSOBOLEW. 'Virchow's Archiv,' vol. 168, p. 91, 1902.
36. GENTÉS. 'C. R. Soc. de Biol.,' vol. 55, p. 334, 1903.
37. KASAHARA. 'Virchow's Archiv,' vol. 143, p. 111, 1896.
38. WRIGHT and JOSLIN. 'Journ. of Med. Research.' Boston, vol. 6, p. 360, 1901. (July-Dec.)
39. V. HANSEMAN. 'Verh. d. Pathol. Gesellsch.,' vol. 4, p. 187, 1901.
40. DIECKHOFF. 'Festschr. gewidm. TH. THIERFELDER,' z. 70. Geburtstag, p. 95. Leipzig, 1894.
41. M. B. SCHMIDT. 'München. Mediz. Wochenschr.,' vol. 49, p. 51, 1902.
42. GUTMANN. 'Virchow's Archiv,' vol. 172, p. 493, 1903.
43. HERTER. 'Medical News.' New York, vol. 80, p. 865, 1902. (Jan.-Jun.)
44. LÉPINE. 'C. R. Soc. de Biol.,' vol. 55, p. 161, 1903.
45. MOURET. 'C. R. Soc. de Biol.,' Ser. 10, vol. 2, pp. 33, 132, 201, 1895.
46. SCHULTZE. 'Arch. f. Mikr. Anat.,' vol. 56, p. 491, 1900.
47. LAGUESSE. 'C. R. Soc. de Biol.,' vol. 54, pp. 852, 854, 1902.

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Assimilation of CO_2 —Influence of Temperature upon; Seasonal Variations in Activity of.

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Respiration (vegetable), Influence of Temperature upon.

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IV. *Experimental Researches on Vegetable Assimilation and Respiration.*III.—*On the Effect of Temperature on Carbon-Dioxide Assimilation.**By GABRIELLE L. C. MATTHAEI, *Fellow of Newnham College, Cambridge.**Communicated by F. DARWIN, F.R.S.*

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* The first two papers of this series emanating from the Cambridge Botanical Laboratory were written by Dr. F. F. BLACKMAN and will be found in vol. 186 (1895) of the 'Philosophical Transactions,' Series B, of the Royal Society.

Section I.—INTRODUCTION AND HISTORICAL SURVEY.

Introduction.

THAT the activity of a plant in the function of assimilation is influenced to a certain extent by the temperature to which it is subjected, has long been recognised, but, in spite of the universal acceptance of this principle, comparatively little detailed study has been undertaken in confirmation of it.

The present investigation was undertaken at the suggestion of Dr. F. F. BLACKMAN and has been carried out in his laboratory. The problem proved to be much more complex than was anticipated and I have to acknowledge, with great pleasure, my indebtedness to Dr. BLACKMAN for frequent valuable advice and several important suggestions.

As the nature of the relation under investigation only unfolded itself gradually in the actual course of the work, I have thought it permissible to expound it in the same way. It will be well, therefore, that the reader should bear in mind that the earlier experiments are, in some cases, of rather a provisional nature though, I trust, well worth permanent record.

This research differs from any which has preceded it on the subject, in the precautions adopted for determining the true internal temperature of the leaf, and in the attention paid to constancy in the environment of the leaves *before* the experiment. A new leaf was used for every experiment, a large number of leaves being kept ready under uniform conditions.

It has always been recognised that to study the effect of a single definite condition on the assimilation or respiration, the other conditions affecting the process must be kept as uniform as possible. Now an additional source of complications has been recognised, for recent investigations by Dr. BLACKMAN and myself have shown that these functions are influenced, not only by the external conditions at the actual time of experiment, but also very considerably, by internal conditions which are the result of the external conditions to which the plant has been subjected for some time previously. Such factors, the effect of which persists, are starvation, excess of nutrition and temperature changes. Thus it is not sufficient to place plants under constant conditions, their whole previous treatment must also have been similar. The ~~non-recognition~~ of this after-effect necessarily vitiates the results of many of the older investigations, in which a return to the normal state was assumed, provided the plant appeared uninjured.

I shall consider here at some length the work of KREUSLER (1) (1888), both because ~~a good illustration~~ of the errors introduced by neglecting uniformity in ~~giving the true relation between temperature and assimilation,~~ because this investigation has hitherto been accepted as

(i) *Consideration of the Researches of Kreusler.*

The greater part of this long and detailed investigation is devoted to the determination of the assimilation and respiration, at various temperatures, of a shoot of *Rubus*. The same shoot was used throughout, and was never removed from the glass-fronted metal box in which the experiment was conducted.

To the back of this box was attached a cell of circulating water by means of which the temperature of the chamber was automatically regulated. This arrangement seems to have been satisfactory only at medium temperatures. Both at the higher and lower limits, a great difference of temperature, sometimes amounting to 5° C., occurred between the back of the chamber and the front.* KREUSLER took the mean temperature as the true one, but, to take an actual case, the effect of keeping some leaves at 0° C. and some at 5° C. can hardly be represented by supposing them all to be at 2°·5 C. At the higher temperatures, where increase of a degree or two might be fatal, this approximation is even less satisfactory.

The light used was that of the electric arc. An air current containing a known percentage of CO₂ was drawn through the plant-chamber, and the amount of CO₂ consumed by the plant was estimated gravimetrically, by absorption of the residual CO₂ by baryta.

Assimilation readings of one hour and respiration readings of several hours were taken at the various temperatures. The assimilation (apparent) and respiration at any one temperature were not estimated consecutively, but whenever convenient—sometimes after the lapse of some days, during which other experiments had been performed on the plant.

KREUSLER summarised his results in the form of a curve,* taking the mean of all assimilation readings at a given temperature, though these were sometimes extremely divergent. He found that the assimilation rose rapidly from 2°·3 C.—11°·3 C. After this point the assimilation increased very slowly, attaining a maximum value at 25° C. Then the assimilatory curve fell again, until the faculty of assimilation was destroyed at the higher temperatures.

The investigations by KREUSLER are an enormous advance on any previous work. His estimations are most careful and no doubt accurate, but his treatment of the plants themselves calls for criticism and especially his non-recognition of the effect of previous conditions.

A leaf, which has been exposed to a temperature above 30° C., may not show any outward marks of injury, but its respiration and assimilation at some medium temperature, such as 18° C., will be very different from what they would have been had it never been exposed to the higher temperature.

As evidence of this point, the following details of an experiment performed at the outset of my own investigations may be given:—

* See PFEFFER (2), 'The Physiology of Plants,' vol. 1, Section 58.

The respiration of twelve cherry-laurel leaves was estimated during 12 hours at $17^{\circ}\text{C}.$ and then the assimilation of one of them was taken at the same temperature, giving a value for the real assimilation of $0.037\frac{1}{2}$ gramme CO_2 per hour. After some hours' exposure to $30^{\circ}\text{C}.$, determinations with the same leaves at $21^{\circ}\text{C}.$ gave a value for the assimilation of only $0.018\frac{1}{2}$ gramme CO_2 per hour. This could not be the true effect, for the amount is about half that obtained at the neighbouring temperature of $17^{\circ}\text{C}.$ Evidently the leaves had suffered by their exposure to the high temperature, and the assimilation could not afterwards rise above a certain limit.

The importance of this experiment lies in its close resemblance to the procedure adopted by KREUSLER, throwing a very unfavourable light on the trustworthiness of his results.

Further we must consider the effect of nutrition on these functions of respiration and assimilation.

It has been shown both by BOUSSINGAULT (3) and by SAROSCHNIKOFF (4), that, if a leaf be allowed to assimilate actively for some time, its power of assimilation may diminish, owing to the accumulation of reserve material, which in some way dislocates the machinery. Such an effect, however, is only produced after prolonged assimilation and would not affect KREUSLER's results, since his assimilation experiments were only an hour in duration.

Much more important is the effect of the nutrition on the respiration. Prolonged darkness produces a steady diminution in the amount of CO_2 given off by a leaf; but if such a starved leaf be allowed to assimilate actively even for an hour or so, the respiration will at once increase greatly, although much additional reserve material may not have been produced. Thus the respiration of a leaf does not depend merely on the temperature, but is greatly influenced by recent assimilation or starvation BORODIN (5).

If we consider the investigations of KREUSLER, we see that he entirely ignored these factors. He assumed that the branch of *Rubus* was throughout in a normal condition and was completely undisturbed by the various changes of temperature or of nutrition which it had undergone. For this reason, he is quite content to estimate the assimilation (apparent) at a certain temperature on one day, and the respiration at that temperature several days later, meanwhile starving and feeding the leaves at will by other respiration and assimilation experiments and exposing them to very various temperatures. Every estimation must have been affected by those which preceded it, and this effect would have been cumulative, making each estimation more untrustworthy than the one before.

A consideration of KREUSLER's numbers will give the best proof of the truth of this criticism.

It will be seen, on looking through the experiments, that only once was a second estimation taken of the respiration at any temperature, i.e., at $33^{\circ}\text{C}.$ on two

consecutive days. The assimilation at 33° C. was also taken twice, on the same days as the respiration. The following are the data obtained :—

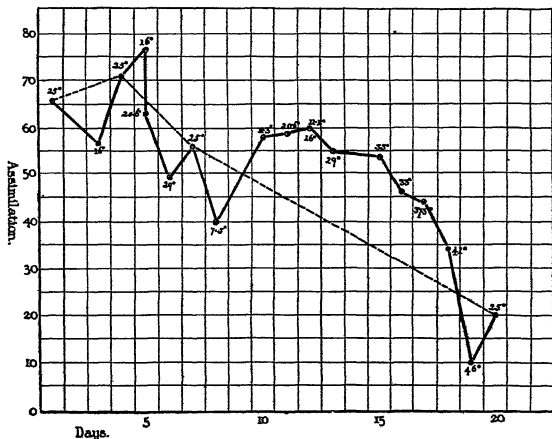
August 8, morning assimilation, 53·4 ; afternoon respiration, 35·4.

August 9, morning assimilation, 43·8 ; afternoon respiration, 30·4.

These numbers are most striking, for although these experiments were performed on consecutive days and the plant was subjected to no injurious temperature in the interval, yet there is a most unmistakable falling-off on the second day, both in the assimilation and in the respiration, showing that the exposure to the temperature of 33° C. on the first day had most certainly affected the vitality of the plant. This effect would most probably persist during further experiments on the branch.

If all amounts of assimilation given in his paper be plotted out (fig. 1) in their

Fig. 1.



chronological order, without regard to the temperatures, a curve is obtained, which is well worth detailed consideration. The general character is most decidedly that of a progressive fall in the amount of the assimilation, notwithstanding that the various temperatures occur in no particular order, but quite capriciously, and that the same temperature is often repeated. Thus, in this series, the amount of assimilation performed by the *Rubus* branch was largely a function of the time during which the experiment had lasted, and was not purely dependent on the temperature.

Moreover, it is most striking that all the highest readings occur during the first few days, and before the plant was subjected to any temperature higher than 25° C. ;

thus the assimilation numbers for the first days are 65, 67, 71, 77, 63; they never rise as high as 60 after the plant has been at the temperature of 29°C ., although the self-same temperatures are again tried. At the beginning also occur the greatest oscillations in the curve, showing that the plant is there able to respond adequately, as regards assimilation, to variations in temperature.

After the eighth day there is a change, the amounts of assimilation at 29°C ., 20°C ., 11°C ., 16°C ., and 33°C ., all have about the same value—indeed, they agree among themselves far better than do readings of the same temperature at different times, from which we again see the influence of the time-effect. Now come the experiments at 33°C ., which have been already mentioned, and which are the prelude to a very rapid fall in the assimilation. The fact that subsequent experiments were all at temperatures above 33°C . easily explains this, for the “after-effect” is greater the higher the temperature. The leaf was kept for 8 hours at 37°C ., and for 8 hours at 42°C ., but nevertheless was still considered to be in a normal state for assimilation at 46°C .

As a finale to the series, the assimilation was again taken at 25°C . and found to be 23.9, in contrast to the values of 65.7, 71 and 56.1 obtained before. This enormous fall must indicate a very great diminution in the vitality of the leaf. KREUSLER, however, was quite content, because it was greater than the assimilation at 46°C ., and showed that the branch could still respond to the stimulus of change of temperature. The four values obtained for the assimilation at 25°C ., if plotted out in their chronological order (as dotted in fig. 1), probably give a very fair indication of the fall in vitality of the leaf.

It will be obvious that KREUSLER's results are not in the least the expression, as he conceived them to be, merely of the effect of the temperature on the assimilation, but are the outcome of the most various causes, so complicated, that it is impossible to extricate from them the simple temperature effect.

(ii) *Consideration of the Researches of Other Investigators.*

The remaining investigations, except those of HEINRICH, amount to little more than a general confirmation of the fact that temperature does affect assimilation and more or less isolated determinations of the lowest temperature at which this function persists.

CHOMON and GRATIOLET (6) (1851) found that for water-plants, the lowest temperature at which assimilation would take place, was $10\text{--}15^{\circ}\text{C}$.

As regards land plants BOUSSINGAULT (7) (1867) found that oxygen was produced by *Pinus laricio* and by certain grasses at temperatures of -0.5°C . to $+3.5^{\circ}\text{C}$., from which he concludes that green trees and the grass of the meadows may very well assimilate throughout the winter.

No importance need be attached to the work of FRANCOPERT (8) (1864), who

attempted to express the connection between the assimilation and the temperature by a mathematical formula. In this research the distinction between the respiration and assimilation was ignored.

BOEHM (9) (1873), in the course of a long paper on intramolecular respiration of leaves, gives the results of a few determinations of the assimilation of *Juglans* at different temperatures, but these are of no great interest.

More important is the work of HEINRICH (10) (1871) on *Hottonia palustris*. His paper embodies the result of a careful comparative study of the effect of different temperatures on the oxygen production of the plant. Unfortunately his results are vitiated by the method he employed, that of counting the number of bubbles evolved in the light from the cut end of the stalk.

Avoidable sources of error, such as the expansion of the gas due to the rise of temperature, movements in the water, &c., are very clearly considered in HEINRICH's paper, together with the precautions taken to overcome them. There are, however, certain defects inherent in this method* which cannot be remedied by any amount of care. The most important of these are the following:—(1) solution of the oxygen in the water, on account of which the absence of gas formation at a low temperature is no proof that assimilation has entirely ceased; (2) the apparent assimilation (*i.e.*, real assimilation less the respiration) only is obtained, this error having most effect the higher the temperature; (3) the same plant must be used throughout since the rate of bubbling depends on the aperture, &c., and thus the experiments are not all performed under exactly similar conditions, since the intermediate treatment of the plant has varied.

HEINRICH found that the assimilation of *Hottonia palustris* increased rapidly with the temperature, reaching its optimum at 31° C., and then fell on a further increase of temperature, until it ceased between 50° C. and 62° C. If the numbers given for the rate of bubbling be erected as the ordinates on the temperature as abscissa, a curve is obtained, which is exactly symmetrical in its rise and fall, and of which the highest point, *i.e.*, maximal assimilation, is the mean point of the temperature range.

This curve is founded on the apparent assimilation, and for this reason only the first part of the curve can represent the effect of the temperature. The higher the temperature the more the results would be vitiated by the neglect of the respiration and by the progressive enfeeblement of the plant.

At the end of the paper, HEINRICH considers the question of the limiting temperatures for assimilation. Intermittent bubbles were formed at 3°·1 C., but a continual stream not below 5°·6 C. This limiting temperature is very much lower than that found by CLOËZ and GRATIOLLET (6), although water plants were used in both cases.

PRJANISCHNIKOW (13) (1876) is the only observer who has taken into consideration

* There is a full discussion of the disadvantages of this method in papers by PFEFFER (11) and REINKE (12).

the effect of varying the intensity of the light at different temperatures. For *Typha latifolia* he found that in direct sunlight the temperatures between 9°·5 C. and 39° C. all gave the same result, but that in diffuse light the assimilation increased in passing from 7–17° C., but fell off in passing from 17–25° C. He concludes that in strong light, when the assimilation is already very great, the temperature cannot increase it further. The falling off of the CO₂ assimilation in diffuse light with temperatures between 17–25° C. he explained by the increase in respiration. These results are quite contrary to those obtained in the present research.

VAN TIEGHEM (14) 1892 in the six lines devoted to this subject in his text-book^{*} expresses the opinion that assimilation bears the same sort of relation to temperature as does respiration. He supports this by quoting four, then (as now) unpublished numbers by BONNIER and MANGIN, which show that the oxygen production of a leafy elm branch augments with increasing rapidity as the temperature rises, at least up to 30° C. Slight and incomplete as it is, this statement gets nearer to the truth than any other published.

There is but one further paper to which reference must be made. In the detailed account given by JUMELLE (15) (1892) of the assimilation and respiration of the lichens, the results of a few experiments performed on individuals of this class at exceedingly low temperatures are given, and, for the purposes of comparison, also a few experiments on phanerogams.

JUMELLE, who was the first to experiment below 0° C., claims to have proved that respiration ceases about -10° C., whereas assimilation persists to -30° C. or even to -40° C. His procedure is, however, unsatisfactory.

A limited amount of CO₂ was admitted into the plant chamber through a long glass inlet tube; samples of the air were then removed through the same tube at the beginning and end of the experiments and were analysed by means of a Bonnier and Mangin apparatus.

In the first place, exception must be taken to the long inlet tube, and to the fact that it serves both to admit the CO₂ and to remove the samples. It might be some time before identity of composition between the air in the chamber and the air in the tube was established by diffusion, and the air removed for analysis would consist mainly of the contents of the tube. Since to indicate respiration the second sample must contain more CO₂ than the first, the existence of a feeble respiration might easily be masked by such an experimental error. On the other hand, a feeble assimilation might be augmented by the same cause.

JUMELLE's results suggest exactly such errors, respiration ceasing at a temperature far above that at which assimilation continued. Blank experiments performed under the same conditions would show at once whether this criticism is just or not. In the absence of any indication as to the probable experimental errors, and considering the impossibility of comparing the relative amounts of respiration and assimilation, we must at present reserve our acceptance of these results.

* * *Traté de Botanique*, vol. 1, p. 179.

In the second place the assimilation result appears to be based on the solitary case of the lichen *Evernia prunastri*. The two lichens of which the respiration was shown to have ceased at -10° C., did not assimilate at -30° C. and -40° C. That they would assimilate at -10° C., JUMELLE assumed but did not prove. The respiration of the two phanerogams, *Picea* and *Juniperus*, which assimilated at -30° C. and -40° C., was not taken at all (at least there is no mention of it in the paper), so that these two cases cannot be taken as evidence of the persistence of assimilation beyond respiration. There remains, therefore, *Evernia prunastri* as the solitary instance in which respiration ceased at -10° C., and assimilation could be detected at -40° C.

Section II.—METHODS AND PROCEDURE.

(i) *Methods of Estimation and Apparatus.*

The apparatus employed in these investigations was designed by Dr. F. F. BLACKMAN, and I must here express my thanks to him for putting it at my disposal.

In this apparatus the amount of carbon dioxide withdrawn by a single leaf from a current of air containing carbon dioxide is taken as the measure of assimilation. The carbon dioxide is estimated volumetrically by absorption in a known volume of baryta, which is subsequently titrated against standard hydrochloric acid.

As a description of the apparatus has, as yet, not been published, I shall enumerate shortly the points necessary to the understanding of the data given later.

Two aspirators are, by an automatic device, kept dropping at exactly equal rates. The single current of air, enriched with the required amount of CO_2 ,* which enters the apparatus is, by their action, divided into two perfectly equal half-currents. One of these currents is led directly through a Pettenkofer tube; the other first passes through the leaf chamber. The difference between the CO_2 shown by the two tubes at the end of the experiment gives the amount assimilated by the leaf. By this exact automatic halving of the current and double estimation, moderate variations in the *absolute* amount of CO_2 supplied cease to be an undeterminable source of error, as the control tube continually gives an exact measure of them.

The whole apparatus contains six pairs of Pettenkofer tubes, and the currents are shifted by clockwork from one pair to the next at intervals of 1, 2, 3 or 4 hours as desired. The apparatus can be left to take care of itself for six consecutive periods, and so assimilation experiments may be carried on continuously day and night. A recording apparatus is attached to the aspirators which gives graphic evidence whether any failure of equalisation has taken place during the time they have not been under observation.

The Pettenkofer tubes that have served their turn may be removed at any

* By the method described in the first paper of this series, 'Phil. Trans.,' B, vol. 186 (1895), p. 496.

convenient time and estimated by washing out their contents into a beaker and titrating with standard HCl and phenolphthalein. They are then refilled with a known amount of standard baryta solution and replaced in the apparatus until the clockwork brings the current round to them again.

Instead of attempting to exclude all traces of atmospheric CO_2 in this procedure, we have tried and found much easier and quite accurate the alternative course of making a definite allowance, "the washing factor," for the CO_2 contamination during washing, titrating and refilling. This has been arrived at by a large number of controls made for the purpose, and is a reasonably constant amount.

The aspirators were always adjusted to the rate of 800 cub. centims. per hour. The percentage of CO_2 contained in the air current varied between .8 and 2.8, the exact amount in any experiment can be calculated from the amount of CO_2 given by the control tube per hour (see p. 87). Care was taken in each experiment that the amount supplied was greatly in excess of the highest amount that would be assimilated in that experiment, in order that there might not be the slightest lowering of the assimilation due to an insufficient supply of CO_2 .

In the data given of all the experiments there will be two columns, one giving the residual amount of CO_2 in the current from the plant chamber, and the other, "the control," giving an amount of CO_2 equal to that supplied to the leaf chamber. The difference between these amounts will be the "apparent" assimilation.*

The successive numbers in any column of control estimations will show a general agreement with slight individual differences, but, as pointed out already, these affect both plant current and control current equally.

I give first the results of two blank experiments, the difference between the amounts of carbon dioxide found in the corresponding tubes is a measure of the total experimental error, including possible slight leaks in the apparatus, titration errors, &c. The procedure exactly resembled that of an ordinary experiment, but

EXPERIMENT I.—January 14–15.

Time.	All the amounts are expressed in grammes of CO_2 .		
	Control tubes.	Tubes from chamber.	Difference.
8.30–10.30 P.M. . .	Preliminary	Preliminary	—
10.30–12.30 A.M. . .	.0307½	.0305½	+ .0002
12.30–2.30 " . . .	.0305½	.0305	+ .0000½
2.30–4.30 " . . .	.0305½	.0304	+ .0001½
4.30–6.30 " . . .	.0303½	.0304½	— .0001
6.30–8.30 " . . .	.0298½	.0299	— .0000½

* The "apparent" assimilation is, of course, numerically less than the *real* assimilation by the amount of respiratory CO_2 that the leaf is to be considered as producing (in the light) at the moment under consideration.

VEGETABLE ASSIMILATION AND RESPIRATION.

EXPERIMENT II.—April 15-16.

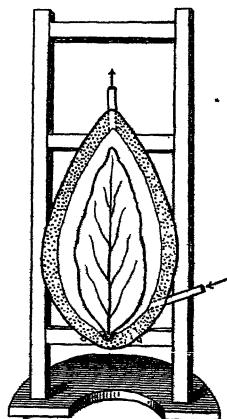
Time.	All amounts are expressed in grammes of CO ₂ .		
	Control tubes.	Tubes from chamber.	Difference.
10.30-12 noon . . .	Preliminary	Preliminary	—
12- 2 P.M.	·0321½	·0322½	- ·0001
2- 4 "	Spoilt	—	—
4- 6 "	·0317½	·0316½	+ ·0001
6- 8 "	·0315	·0310½	+ ·0004½
8-10 "	·0309	·0313	- ·0004
10-12 midnight . . .	·0312	·0315	- ·0003
12- 2 A.M.	·0315	·0315½	- ·0000½
2- 4 "	·0317½	·0318½	- ·0001
4- 6 "	·0316	·0317	- ·0001
6- 8 "	·0313½	·0312½	+ ·0001
8-10 "	·0313	·0312½	+ ·0000½

there was no plant in the chamber. It will be seen that the numbers in the "difference" column agree much more closely than the absolute amounts of CO₂, proving the exact halving of the current.

The small value of the experimental errors shown in these experiments, and the good agreement of the assimilation numbers subsequently to be given, prove that a very fair degree of accuracy in the CO₂ determinations has been obtained.

This method of continuous observation possesses most important advantages. Among others every determination of the assimilation depends not on a single estimation, but on four or five consecutive estimations made under unchanged conditions, and this is of immense value in an investigation of this nature in which the experimental difficulties are great, and the factors which may affect the result numerous.

For the assimilation experiments, a specialised form of flat glass leaf-chamber was employed. This consisted of a broad rim of iron somewhat larger than the outline of the leaf, with an exit tube through the top and an inlet tube through one side near the base. A plate of glass fastened permanently to one surface of this rim by wax formed the front of the chamber. The back was formed by a similar piece of glass which was waxed on to the other surface of the rim each time after the leaf was put in, care being taken that the whole should be quite air-tight. This made a shallow chamber about 5 millims.



Leaf-chamber on stand.

deep, provided with inlet and outlet tubes. The content of such a chamber is not more than 40 cub. centims.

The chamber was fastened vertically to a brass stand. As the chamber was very shallow, the leaf remained flat and erect, lightly held between the back and the front glasses. The stalk dipped into a small amount of water.

The leaf chamber with its stand was placed in a large rectangular copper bath, filled with water, the front of the bath having a glass window, so that the leaf may be illuminated.

For respiration experiments involving several leaves, larger vessels of metal were employed.

(ii) *Regulation of the Temperature.*

For high temperatures the bath was heated by a small flame controlled by a thermostat. Temperatures near that of running water ($5-14^{\circ}\text{C}$. according to the time of year) could be obtained by allowing water to circulate freely through the bath. Intermediate temperatures, *i.e.*, those between those of running water and the higher temperatures, could be obtained by combining both the flame and the current of water.

By these means the temperature of the bath could be maintained very constant, the variation during any individual experiment seldom amounting to more than $0.2-0.4^{\circ}\text{C}$. of a degree.

The air current before entering the leaf chamber always passed through a long spiral tube immersed in the bath, in order that it might attain the exact temperature of the bath.

There was some difficulty in keeping the temperature of the bath constant in the neighbourhood of 0°C . and below it, especially on account of the proximity of the incandescent burners used as source of illumination.

For temperatures just above 0°C ., the bath was filled with a mixture of ice and water, for temperatures below 0°C ., with a mixture of ice and salt solutions of various strengths. Ice was continually added and solid salt from time to time to remedy the dilution produced by the ice. Although the ice floats at the top, a uniform temperature throughout the bath was obtainable by the energetic use of an automatic air-stirrer.

The continual addition of ice and salt soon produces a slight opalescence in the bath-solution in spite of washing the ice and filtering the salt solution, but a consideration of Table IV. shows that the effect on the results has been unappreciable, as there is no falling-off during the course of an experiment, although the opalescence always increased with the time.

These experiments at low temperatures necessitated constant attention; it was impossible to leave them unwatched for more than 10 minutes: as, unless the amount

of ice in the bath was very carefully regulated, the temperature was liable to change. For this reason I have at some of these temperatures contented myself with three readings instead of taking the usual four or five.

(iii) *Sources of Light.*

At the beginning of the investigation ordinary incandescent gas-light was employed. Ultimately this light was found insufficient, and the Keith high-pressure incandescent light was employed.

With the ordinary burners the gas pressure was kept constant at about 1 inch of water by means of a Moitessier regulator. Mantles of one make only were employed, either a new one for each experiment, or, where stated, one for a series of experiments.

The weakest light used, that obtained when the front of the mantle was 130 millims. from the leaf, is termed light of unit intensity.* By varying the distance between the leaf and the burner according to the law of inverse squares other intensities were obtained. A limit was imposed on the increase in the light which could be thus obtained, by the necessity of leaving a certain amount of space between the front glass of the leaf chamber and the window of the bath to insure free circulation of the water and consequent uniformity in the temperature. The greatest amount of light obtainable in practice from the single incandescent burner by increasing its proximity to the leaf was a light of sixfold intensity.

A somewhat increased intensity of light was obtained by using *two* burners side by side, but these could not be placed nearer to the window of the bath than 31 millims. (for fear of cracking it). The distance from the leaf was now 55 millims., and the intensity thus obtained was eightfold.

In the Keith burner the gas is supplied by a self-regulating water-motor under a very constant high pressure (8 inches of water), and this gives an extremely steady light.

To obtain a very intense light from this apparatus, three burners, arranged on a triangular plan, were employed. Two were placed side by side, in a plane parallel to that of the window of the bath and the third just behind, filling the small gap between them. These three burners were mounted on a brass stand, so that their relative positions remained unchanged, and they could be used as a single source of light.

The light of the three burners was compared, by means of a grease-spot photometer,

* All the intensities of light subsequently used are expressed in terms of this unit intensity by making due allowance for alterations in distance and differences in the burners employed. Of course with a compound source of light such as an incandescent mantle, such a comparison of intensities makes no pretence to accuracy, but may serve as a rough guide to the relative amounts of light necessary for maximal assimilation at various temperatures, and is absolutely necessary for convenience of reference.

It will soon also be made clear that knowledge of the exact intensity of light being used is not of critical importance in these investigations.

with a single ordinary incandescent burner, and it was found to be about six times that obtained from the single burner.

This relation has been adopted as the basis for comparing the intensities of the light, obtained in the later experiments, with those of the earlier ones.

These burners, although so excellent a source of light produce, however, a very large amount of heat. The simple system of circulating water employed before was quite inadequate to keep down the temperature of the bath. It was necessary to absorb more effectually the heat radiations before they reached the bath, by means of a special water-screen.

The screen employed consisted of an erect glass plate down both surfaces of which a thin sheet of water flowed rapidly. This was placed a few millimetres away from the window of the bath and the burners could be brought so close to the screen that only 24 millims. intervened between them and the window. With this arrangement the temperature of the bath itself could be kept constant at as low a temperature as 11°C .

With the ordinary incandescent burner the heat radiation was not sufficient to produce in the leaf, by reason of its greater absorptive power for radiation than that of water, any appreciable rise of temperature over the temperature of the bath. This was proved by wrapping the bulb of a delicate thermometer up in a piece of leaf, placing it at the proper distance from the light and comparing its readings with those of a similar thermometer with uncovered bulb. The excess temperature of the wrapped-up thermometer was not more than one-tenth of a degree even with the bath at the low temperature of -6°C .

With the powerful Keith burners, however, the effect was very different and the wrapped-up thermometer showed an excess temperature of 6° or 7° (tested with the bath at 11°C . and at 32°C .).

It was, therefore, quite obvious that here the temperature of the bath afforded no clue to the true temperature of the leaf, and that in order to obtain the true relation between assimilation and temperature when employing an intense light, it would be necessary to determine the internal temperature of the leaf during each assimilation experiment.

This, although introducing great additional complexity into the inquiry, was accomplished by employing thermo-electrical methods. A full description of these is

They showed that the temperature of the leaf, when exposed to powerful radiation may be 10° or more above the temperature of the bath.

(iv) Procedure.

Very suitable material for these investigations was yielded by the leaves of cherry

laurel, *Prunus Laurocerasus*, garden variety *rotundifolia*. To insure similarity in environment the leaves were always cut from the same side of one particular bush. Great care was taken to select them as far as possible of the same size and development, in order to reduce individual variation to a minimum.

Stress has already been laid on the importance of keeping these leaves under exactly similar conditions for some time before use. That the realisation of this ideal is attended with a certain amount of difficulty, the following considerations will show.

Since the effect of keeping isolated leaves in the dark, is to produce a continuous fall in the respiration, the amount of CO_2 given off by any leaf would vary with the number of days that it had been picked. On the other hand any increase in nutrition, *i.e.*, exposure of the leaf to a bright light, causes a decided increase in the respiration.

It was also impossible to use leaves straight from the tree; for it had been found (unpublished investigations by Dr. BLACKMAN and myself) that the respiration of a leaf during the first 24 hours after isolation might be extremely irregular. It was, therefore, necessary to wait at least 24 hours after cutting, before using the leaf, and yet to prevent either a diminution or increase in the respiration.

These demands were fulfilled by keeping the leaves with their stalks in water in covered beakers, exposed to diffuse light. Presumably they were just able to assimilate sufficiently to cover the loss in reserve material due to respiration. A fairly constant temperature was maintained, averaging between 10°C. and 14°C. at different times.

Good evidence that by this arrangement leaves could be kept in a uniform condition for several days, will be found among the records of the respiration experiments (Table I.). For instance, there are two determinations of the respiration at 14°C. These were both performed on the same set of leaves, which in the interval (a week) had been kept under the above-mentioned conditions. The agreement between the two sets of readings is most satisfactory.

The two determinations of the respiration at 33°C. were taken from two different sets of leaves, kept for varying lengths of time in the same way. Here also there is practically no difference in the readings.

In the assimilation experiments a fresh leaf was employed for each experiment. This, as mentioned before, constitutes one of the fundamental points of difference between this investigation and those which preceded it.

To avoid any loss of water-content by excessive transpiration during the experiment, the leaf stalk, with its surface freshly cut, was placed so as to dip under the water at the lowest point of the chamber. A comparison of the weights of the leaves before and after the experiment shows that this precaution was quite sufficient.

The general procedure of an experiment was as follows—A leaf (chosen from those kept under the above-mentioned conditions) was weighed and the area measured by means of a polar planimeter. It was then sealed up in the chamber and the latter was placed in the bath, which was already at the required temperature. The distance

between the source of light and the chamber was adjusted, the aspirators, clockwork CO₂ generator, &c., started and adjusted.

The currents were allowed to run for 1½–2 hours before actual readings were taken, to render the conditions, such as CO₂ content of the chamber, &c., constant. Consecutive 2-hour readings were then instituted, and at the end of the experiment the leaf was removed, weighed, and any change in the appearance noted.

It may be of interest to give here two experiments which illustrate well the need for uniformity in the condition of the leaves before experiment. In both the cases given below the leaves were picked during a sharp frost and were used immediately for assimilation experiments at temperatures of 14° C. and 18° C. It will be seen that the value obtained for the assimilation rises throughout the experiment. This may be attributed to the gradual opening of the stomata from the winter closure, under the influence of the light and warmth, or, perhaps, partly also to the slow recovery from "assimilatory inhibition," that had been brought on by the continued frost, see EWART (16). In either case the effect lasted for 10 or 12 hours. Subsequent experiments on leaves, that had not been exposed to frost, showed that the highest number obtained represents the normal assimilation.

These experiments also form a good illustration of the value of the system of taking continuous readings. Any one of the readings singly would have been very misleading.

EXPERIMENT III.—December 17–18. 14°2 C.

Weight before and after experiment.	Temperature.		Time.	All amounts are expressed in grammes of CO ₂ .			
	Area.	Time.		Temp.	CO ₂ supplied.	CO ₂ unassimilated.	Apparent assimilation.
					Control tubes.	Tubes from chamber.	Difference.
1st weight, 1.38 grms. 2nd weight, 1.40 "	9.30 P.M. 12.15 A.M.	14°3 14°2	9-11 P.M. 11- 1 A.M.	Preliminary •0297½	Preliminary •0294	— 0003½	
Area, 47.0 sq. centims.	9.30 A.M.	14°2	1- 3 "	•0297½	•0277	•0020½	
			3- 5 "	•0301	•0269½	•0031½	
			5- 7 "	•0300½	•0266½	•0034	
			7- 9 "	•0297½	•0256	•0041½	

EXPERIMENT IV.—December 9–10. 18° C.

Weight.	Temperature.		Time.	All amounts are expressed in grammes of CO ₂ .		
				CO ₂ supplied.	CO ₂ unassimilated.	Apparent assimilation.
	Time.	Temp.		Control tubes.	Tubes from chamber.	Difference.
Weight, 1.68 grammes	9 P.M.	18.1	9–11 P.M.	Preliminary	Preliminary	—
			11– 1 A.M.	.0320	.0284	.0036
			1– 3 "	.0322	.0285	.0037
			3– 5 "	.0325	.0277½	.0047½
			5– 7 "	.0319½	.0271	.0048½
	9 A.M.	18.2	7– 9 "	.0322	.0270½	.0051½

Section III.—EXPERIMENTS AT LOW AND MEDIUM TEMPERATURES.

(i) *Respiration.*

Since the determination of the real assimilation of a leaf necessitates a knowledge of the respiration at the time, it was important to determine this under conditions exactly similar to those to which the leaves were subjected in the assimilation experiments. The respiration at the temperatures considered in this section is small in comparison with the assimilation, and no critical work in this regard is required. At all temperatures below 0° C. the respiration could be neglected, for it was clear from the determinations at 5° C. that below 0° C. the amount for a single leaf would come within the experimental errors.

The procedure adopted in determining the respiration at medium temperatures was similar to that employed in an assimilation experiment, with the exception that the two currents were in this case freed from CO₂, and an estimation was made of the amount of CO₂ added to one of them in a given time by the plant. Since the respiration of a single leaf was very small, several leaves were taken for each determination, the number of leaves being regulated to give an amount of CO₂ convenient for measurement.

The results obtained in these determinations of the respiration are given in Table I. The consecutive readings in any one experiment show good agreement, so do also the duplicate experiments. Moreover, when the respiration of a single leaf is calculated from these results, small inaccuracies in the actual determinations are very much reduced. Even the slight fall in respiration, which normally occurs during a prolonged experiment in the dark, is practically eliminated in the reduced numbers.

A curve showing the respiration at different temperatures is given in fig. 2, p. 65. The numbers employed in its construction are the average values obtained from each experiment. Here the respiration, in all cases, has been calculated for a constant weight of 2 grammes.

The assimilation results are calculated in all cases for a leaf surface of 50 sq. centims. The weight of this is approximately 2 grammes, so that the respiration and assimilation results are more or less comparable.

(ii) *Experiments with Unit Intensity of Light.*

Full details of the experiments to be considered under this head are given in Tables II., III. and IV. at the end of the paper. The various columns explain themselves, and but few additional remarks are needed.

The temperature indicated by the thermometer in contact with the leaf chamber was noted at intervals throughout the experiment. These readings, with the time at which they were taken, are given in the second and third columns. In the low temperature determinations, where the experiment was watched the whole time and the temperature regulated by the amount of ice added, this seemed superfluous, and more general statements are given.

The eighth column shows the respiration (deduced from the results given in Table I.) for the weight of the individual leaf used in the assimilation experiment. Since all the cells take part in the respiration, it seemed more correct to refer the respiration to the weight of the leaf than to the area of leaf surface as was done by KREUSLER (1). The assimilation, on the other hand, varies with the leaf surface.*

The only column which contains numbers directly comparable with those obtained in other experiments is the last. Here allowance has been made, not only for the respiration, but also for the leaf area. The assimilation for 50 sq. centims. and 1 hour has been taken as the standard.

Table II. gives the results of assimilation experiments performed between the temperatures 14° C. and 33° C.

Similar experiments are described in Table III., but here the temperature limits are 5° C. and 25° C. The results of experiments at temperatures between -6° C. and +8°·6 C. are given in Table IV. As mentioned before, the respiration below 0° C. could be neglected. A slight difference in the procedure was necessarily adopted, and no water was placed in the leaf chamber in any of the experiments at temperatures below 0° C.†

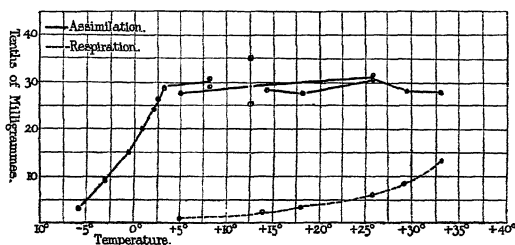
* Where the respiration at a particular temperature has not been found experimentally, the amount has been calculated from the respiration curve given in fig. 2.

† The following experiment shows that this lack of water had no inhibitory effect (at low temperatures)

A leaf was set up in the chamber in the usual way, but without water. Three readings of the assimila-

The detailed results given in these tables are represented in the curve (fig. 2). The latter embodies the mean result of every experiment. Each point represents the average of the readings obtained in a single experiment only.

Although, without exception, every experiment made is represented in fig. 2, yet there are only two numbers which are anomalous, namely, two experiments at 14° C. (located by error at $12^{\circ}5$ in the figure) and of these the mean falls perfectly into line.



tion were taken at the temperature of $5^{\circ}8$ C. Water was then introduced into the chamber, and, after allowing the conditions to become constant, readings were again taken at the same temperature. The result was conclusive; no rise at all was produced in the assimilation, rather there was the inevitable falling off, due to the length of the experiment.

EXPERIMENT XXV.—February 18. $5^{\circ}8$ C.

Weight and area.	Temperature.		Time.	All amounts are expressed in grammes of CO ₂ .		
	Time.	Temp.		CO ₂ supplied.	CO ₂ unassimilated.	Apparent assimilation.
				Control tubes.	Tubes from chamber.	Difference.
1st weight, 1.57 grms.	10.45 A.M.	5° 6	10.15–11.45 A.M.	Preliminary	Preliminary	—
	11.45 "	5° 7	11.45– 1.45 P.M.	•0309	•0256½	•0052½
Area, 43.0 sq. centims.	2.30 P.M.	5° 7	1.46– 3.45 "	•0311½	•0264	•0047½
	3.38 "	5° 8	3.45– 5.45 "	•0311½	•0271½	•0040
			Water added			
			5.45– 6.36 P.M.	Preliminary	Preliminary	—
	6.45 "	6° 0	6.36– 8.36 "	•0315½	•0275½	•0040
			8.36–10.36 "	•0313	•0272½	•0040½
	12.10 A.M.	6° 1	10.36–12.36 A.M.	•0310	•0273½	•0036½

The curve is made up of three parts, denoting the result of the three series of experiments given in Tables IV., III. and II. respectively. The temperature range of each series has been so chosen as to overlap that of the next. This arrangement enables a direct comparison to be made between them, and gives confirmatory evidence for part of the temperature range.

The slight dislocation between the parts where they overlap is most probably an expression of the difference between the light produced by the two incandescent mantles employed. Thus this amount is shown to be not of sufficient magnitude to cause any effective disturbance of the curve.

The first point to be noticed is that assimilation can be detected as low as -6°C . Previous workers with less exact methods have not detected it below 0°C .* *e.g.*, CLOEZ and GRATIOLÉ (6), $10-15^{\circ}\text{C}$. for *Potamogeton*, &c., HENRICH (10) $2-4^{\circ}\text{R}$. for *Hottonia*, and BOUSSINGAULT (7) $+0.5$ to $+3^{\circ}\text{C}$. for *Pinus laricio* and grasses. This is due partly to the methods of observation, and in some cases to the choice of plants. Delicate water-plants would most probably cease to assimilate long before the hardy evergreen cherry laurel. It is very probable that cold-loving plants, such as conifers, lichens, &c., may assimilate at even lower temperatures.

The next point to be noticed is the sharp change in the character of the curve at $+3^{\circ}\text{C}$. There is an extraordinary uniformity in the assimilation from $+3^{\circ}\text{C}$. to 33°C . Individual differences in the readings of any one series are hardly greater than the experimental error obtained in the actual experiments, and this, considering that separate leaves were used each time, and that slight variations in the light, &c., were possible, is most satisfactory and completely demonstrates the validity of the methods adopted in the treatment of the leaves.

Below $+3^{\circ}\text{C}$. we find the assimilation suddenly decreasing very rapidly with the temperature, until, at -6°C ., the leaf has practically lost this faculty. This curve of decrease of the assimilation is well established, for the intervals of temperature between the successive determinations are extremely small. There are no less than seven separate determinations in the range from -6°C . to $+3^{\circ}\text{C}$. Since the assimilation was changing so rapidly, a large number of experiments at slightly different points, seemed more conclusive than several determinations at one or two, more widely separate, temperatures.

This marked contrast between the nature of the assimilation curve above and below 3°C . is so remarkable that it suggested some influence other than the direct effect of the temperature. On reviewing the factors influencing assimilation which might possibly be affected by a low temperature, there seemed but one probable one, partial closure of the stomata. This might well be brought about by intense cold, resulting in a very reduced value for the assimilation. A separate experiment was, therefore, undertaken to test the validity of this hypothesis.

* As has been shown in the Introduction, it is difficult to accept JUMELLE's results at -30°C . and -45°C .

If artificial openings be made in a leaf with closed stomata a great increase in the assimilation results. The assimilation of a certain leaf was taken at $-3^{\circ}6$ C. After two readings the leaf was removed and cut in 40 places pinnately with a sharp scalpel. The leaf was then replaced in the chamber and readings were again taken. Had the

EXPERIMENT XXVI.—February 24 and 25, 1902. $-3^{\circ}6$ C.

Weight and area.	Temperature.	Time.	All amounts are expressed in grammes of CO_2 .		
			CO_2 supplied.	CO_2 unassimilated.	Apparent assimilation per 2 hours.
			Control tubes.	Tubes from chamber.	Difference.
Weight, 1.78 grms. Area, 48.7 sq. cms.	$-3^{\circ}6$ C. to $-3^{\circ}8$ C.	10.15–11.45 A.M.	Preliminary	Preliminary	—
		11.45– 1.45 P.M.	·0303	·0282	·0021
		1.45– 3.45 "	·0300½	·0283½	·0017
		3.45– 5.45 "	·0312½	·0297	·0015½
		Leaf in chamber in light during night; temperature about 7° C. At 11 A.M., cut 40 cuts pinnately.			
	$-3^{\circ}6$ C.	11.15–12.45 A.M.	Preliminary	Preliminary	—
		12.45– 4.55 "	·0606	·0587½	·0009
		4.55– 6.55 "	·0288	·0277½	·0010½

stomata been closed there must have resulted a great increase in the assimilation. This was not the case; in fact there was a decrease owing to the duration of the experiment. The rise in the respiration resulting from the wounding of the leaf could introduce no error, since at so low a temperature the respiration even if doubled or trebled would still be negligible.

As a contrast to the above an experiment may be given in which cutting produced an increase in the assimilation. A leaf dried over calcium chloride assimilates very feebly. On cutting, the CO_2 absorption became very much greater, showing that the small amount obtained before was due to closure of the stomata (see Exp. XXVII.).

As regards the change in form of the curve (fig. 2), Experiment XIX., Table IV., in which the temperature was gradually raised during a reading, gives a very good demonstration of the fact that at $+3^{\circ}6$ C. the value of the assimilation is already quite the same as that represented by the level portion of the curve, while numerous other experiments show that this is not the case for the values below $+2^{\circ}$ C.

It was impossible to believe that the influence of the temperature on the assimilation really changed its character in the sharp and sudden manner the curve suggested, and yet the true interpretation was not at first sight obvious.

EXPERIMENT XXVII.—February 21.

Weight and area.	Temperature.		Time.	All amounts are expressed in grammes of CO ₂ .		
	Time.	Temp.		CO ₂ supplied.	CO ₂ unassimilated.	Apparent assimilation.
				Control tubes.	Tubes from chamber.	Difference.
Original weight, 1.98 grammes; dried over CaCl ₂ , weight, 1.75 grammes	3.45 P.M.	9° 2	2.50- 4.50 P.M.	Preliminary	Preliminary	—
			4.50- 6.50 "	·0305½	·0301½	·0004
			6.50- 8.50 "	·0314	·0309½	·0004½
	9 "	8·0	8.50-10.50 "	·0306½	·0304½	·0002
			Cut at 11.30 "	—	—	—
Area, 53.1 sq. centims.	11 "	8·2	—	—	—	—
	1 A.M.	8·2	12.6 - 2.8 A.M.	Preliminary	Preliminary	—
			2.8 - 4.8 "	·0310	·0259	·0051
			4.8 - 6.8 "	·0307½	·0254½	·0053
	11.15 "	7·6	6.8 - 8.8 "	·0310	·0253	·0058

A very valuable suggestion was then made to me by Dr. BLACKMAN, which provided the key to the whole problem.

He suggested that only the first sloping part of the curve corresponded to a condition of things in which the amount of assimilation was really a direct function of the temperature, and that the level second part was simply an expression of the full amount of CO₂ that the energy of the light provided was able to decompose. This amount is governed by thermo-chemical considerations, and is not increased by raising the temperature.

The limiting assimilation maximum, fixed directly by any given temperature, can then only be arrived at when the light is adequate for the decomposition of the amount of CO₂ in question, and when, also, of course, that amount of CO₂ is freely available. Therefore, to reach these maxima for the higher temperatures, more light must be employed, and the evidence that is to be looked for to show that the limit is reached, and that the light really is sufficient, will be of the nature of showing that, at the given temperature, increase of light no longer augments the assimilation.

These considerations are fully borne out by experiment, and it is found that, measuring the assimilation over the whole temperature range in a low intensity of light, gives a curve (as in fig. 2), with its changing point, at a low temperature; working with a somewhat higher intensity, gives the change at a higher temperature (as in fig. 3), while, if the light is very intense, the change to the level portion comes very high up, or, indeed, not at all (fig. 5), the curve being instead

interrupted by the effect of sub-fatal temperature. At high temperatures, then, it is only with such intense light that the pure temperature effect can be evaluated.

It is interesting to note that the final curve put forward by KREUSLER is really of the imperfect compound nature indicated. His procedure, however, was not perfect enough to bring out the changing point as a sharply-defined effect. It looks in his curve merely like a slow change in steepness, such as might be a direct temperature effect. The more accurate methods here employed brought out the changing point as so sudden and abrupt an effect that it could not be overlooked.

(iii) *Experiments with Various Low Intensities of Light.*

These experiments were undertaken to prove the truth of the hypothesis just brought forward.

At three different temperatures— $+0^{\circ}4$ C., $+9^{\circ}$ C., $+11^{\circ}$ C.—the assimilation with the unit intensity of the light (*i.e.*, that used in the previous experiments) was determined and then the light was increased until no further increase was produced in the assimilation. A different limit for the assimilation was found in each case, and, to reach it, a greater intensity of light was necessary the higher the temperature. In the experiments at 25° C. this limit was not reached even with a sixfold intensity of light.

Table V. embodies the results of these experiments. The arrangement is, in the main, the same as that in the preceding tables. Column 1 contains a statement of the relative intensities of the light. These were obtained by shifting the leaf chamber in relation to the window of the bath, while the position of the burner was unchanged.

Notifications of the temperature in some of the experiments in Table V. are very few, because water running rapidly through the bath was used, and this gives a very constant temperature.

Readings with the leaf chamber in the "unit light" position were first taken, then the chamber was moved into the position required for the greater intensity and determinations were again made, after a short preliminary to obtain equilibrium of conditions. It was found impracticable to determine the effect of more than two different intensities of light on the same leaf.

Table VI. gives a summary of these results. Here both the apparent and the real assimilation* for 50 sq. centims. and 1 hour are given, being the average of all the individual results of Table V., also the ratios between the amounts of the real assimilation at double, fourfold and sixfold intensities, to that obtained with light of unit intensity.

* The real assimilation has been obtained from the apparent in the same way as before by using the results given in Table I.

If we now consider the results under the heading of "Real Assimilation," we find that the amount of assimilation obtained with light of unit intensity is approximately constant* at all temperatures except the lowest. Here, as we should expect from the previous results, the amount is lower. Not even all the amount of light represented by $L.In. = 1$ can at the low temperature be utilised.

When the intensity is doubled, the assimilation at $0^{\circ}4$ C. is unaltered, but an increase is produced at the other temperatures, numbers almost identical being obtained for all of these. When four times the light is used we find a similar phenomenon. The assimilation at 9° C. is no greater than that corresponding to twice the light, for the temperature exerts its limiting effect and the leaf can assimilate no more. At 11° C. a higher number is obtained, which, however, is not so great as that obtained at 25° C., showing that the leaf can make use of more light than is given by $L.In. = 2$, but it cannot use all that of $L.In. = 4$. Sixfold light gives practically the same result as fourfold light for 11° C., showing that the maximum has been attained.

Thus at the temperatures of $0^{\circ}4$ C., 9° C. and 11° C., by sufficiently increasing the light, a limiting amount of assimilation has been obtained, different in each case.

Fig. 3.

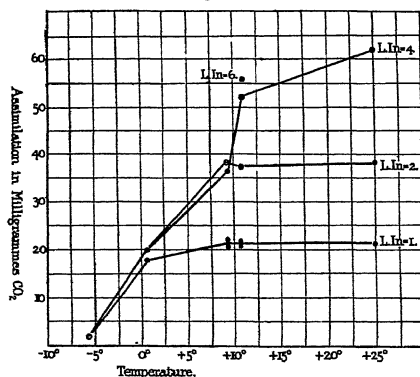


Fig. 3 represents graphically the results of Table VI. Three different curves are obtained, one with the unit intensity of light, one with twice the light, and one, the true assimilatory curve for the temperatures from -6° C. to $+11^{\circ}$ C., with fourfold light. The truth of the hypothesis is, therefore, well-established.

* Experiment XXXVI. must be considered merely as regards the ratio yielded. The results obtained for $L.In. = 1$ and $L.In. = 4$, though harmonious, are far too small in comparison with the other experiments, indicating that the leaf was probably in an abnormal condition.

In considering the ratios given in the last four columns, it is of interest to note that, although the assimilation is not maximal, light of double the intensity does not produce fully double the assimilation. It would seem as if, a little below the limit, the leaf could not respond completely to the light.

Thus the law that the assimilation varies with the intensity of the light appears only to be true for degrees of illumination well removed from that necessary to produce the maximal amount. This needs further investigation, but some determinations by REINKE (17), are, to a certain extent, confirmatory. He found that, after a certain limit, increase in the intensity of the light produced no effect on the assimilation, and that, just below this limit, the increase in the assimilation was not proportional to that of the light.*

Section IV.—EXPERIMENTS AT HIGH TEMPERATURES.

(i) *Respiration.*

Before dealing with the actual assimilation numbers obtained at these temperatures it will be necessary to again consider shortly the question of the respiration. The higher the temperature the more important a knowledge of the true value of this function becomes.

Unfortunately the question of the behaviour and value of the respiration during exposure to high or even to comparatively high temperatures is very complicated.

A separate inquiry was made into the general behaviour of the respiration of leaves under various conditions at high temperatures. Many interesting results were obtained, having an important bearing on our whole conception of the function of respiration and its reaction to changes in condition. A full account of this investigation will be published separately, but it will be necessary to state here very shortly some of the results that bear on the present question.

The value of the respiration changes rapidly with the time of exposure, and consecutive 2-hour estimations of the respiration of the single leaf, prove that, instead of forming a smooth falling curve, the CO_2 produced by the single leaf shows a large number of oscillations, much too great to be due to experimental errors. Moreover, the individual variation in the respiration of the leaves under similar external conditions is considerable, due, no doubt, in great measure, to differences in internal condition, such as amount of carbohydrates present, &c. Such individual variations and oscillations occur at the low temperatures also, but are so small in amount that they are not greater than the experimental errors. It is, therefore, quite impossible to predict with certainty the value of the respiration of a leaf at any particular moment by observations on another leaf under the same external conditions.

* It will have been observed that the assimilation obtained with unit light is much lower than that obtained in the earlier experiments with the same light. This is due to a seasonal change in the condition of the leaves on the tree, and will be dealt with later, see pp. 82 and 83.

However, these factors, although exerting an appreciable influence, are nevertheless insignificant when compared with the enormous effect produced on the respiration by assimilation. *The respiration is continually changing during an assimilation experiment by virtue of the assimilation*, and may be doubled by an exposure of 4 or 5 hours to light and CO_2 . This is due doubtless to the production of a fresh supply of carbohydrates, but the rise produced is not entirely proportional to the amount of CO_2 decomposed.

Thus as it is at present impossible to determine directly respiration in presence of assimilation, we have no means of obtaining the exact value of this quantity, and we are obliged to make an approximation.

It will be obvious that, if the respiration of the leaf be taken in the dark, before the assimilation experiment, too low a value is obtained; if taken after the assimilation, the value tends to be too high. By taking the mean of these two determinations, we must, however, obtain an average value which cannot be very different from the real value during the assimilation, and is certainly accurate enough for the purposes of these experiments.

This is the plan that has been adopted in the experiments at high temperatures given in Table VII. In the experiments of Table XI. it was impracticable to determine the respiration twice owing to the complexity of the experiment and the necessity of completing this series of experiments within a short time.* Under these circumstances the respiration was taken *after* the assimilation only. The value employed for the respiration is, therefore, admittedly too high, but the difference from either the average value or the true value is not sufficient to seriously affect the values of the real assimilation nor to invalidate the form of the curve.

(ii) *Experiments with Eight-fold Intensity of Light.*

In these experiments the source of light consisted of two ordinary incandescent burners placed side by side at a distance of 55 millims. from the leaf, giving a light of eight times unit intensity.

The leaf was set up in the usual manner, and, after an hour's preliminary, two 2-hour estimations of the respiration were made in the dark. The leaf was then exposed to the light, and several 2-hour estimations of the apparent assimilation taken. The chamber was then darkened and the respiration again determined.

~~In order to reduce as much as possible the necessary preliminary between exposure to light and darkness, the current containing CO_2 was drawn through the apparatus during the whole time, and thus, a departure from previous procedure, the respiration was estimated as an addition to the current of CO_2 passing through the chamber.~~

Determinations of the assimilation were made at temperatures of 11°C ., $25^\circ 4 \text{C}$.,

* The condition of the leaves was rapidly changing owing to a change in the season, see p. 82.

32°·1 C., 38°·3 C., 40°·9 C., and 42°·9 C. (Table VII., fig. 4). The results of the two experiments made at each temperature are in good agreement.

In Experiment XXXVII. the respiration was not estimated, but was calculated as before, from Table I., it being impossible to determine accurately, with the apparatus employed, the CO_2 produced at 11° C. by a single leaf in 2 hours.

In this experiment after three 2-hour determinations of the assimilation with the light of eight times unit intensity, the light was changed to the single incandescent burner at a distance of 42·5 millims. giving an intensity of light represented by 4·6 times unit intensity. No change, however, was produced in the amount of the assimilation, showing that the lesser intensity of light is sufficient for maximal assimilation at this temperature.

That this is so may also be shown by reference to the other experiments in the series. At 11° C. the amount of assimilation obtained was ·0068 gramme per hour. By raising the temperature to 25° C., the value of the assimilation was increased to ·0126 gramme per hour with no alteration in the light provided. It is clear, therefore, that, since the light was sufficient to decompose ·0126 gramme of CO_2 per hour, the small amount of assimilation obtained at 11° C. was not due to insufficiency of illumination. Since the leaf has therefore more than enough light and CO_2 , the value obtained for the assimilation must represent the maximal assimilation of the leaf at 11° C. It is impossible to obtain a greater assimilation except by raising the temperature.

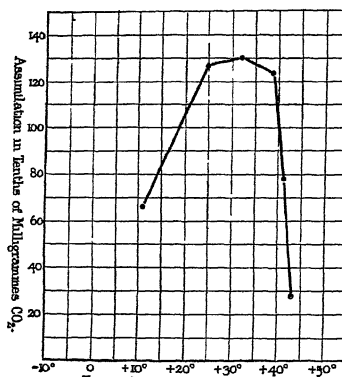
Let us consider now the case of the other experiments. Since the value obtained throughout the range of temperature 25–38° C. is the same, it is clear that this is an expression of the amount of CO_2 which can be decomposed by the light supplied, and bears no relation to the temperature. The leaf assimilates as much as the illumination provided allows, if it is not limited by the temperature it is so by insufficiency of the light.

At temperatures higher than 38° C. the assimilation falls much below the limit imposed by the illumination, showing that here the amount is limited by some condition other than that of the light. Rise in temperature has begun to exert an injurious effect. At 45° C. the influence of the temperature alone is sufficient to kill the leaf. It is not surprising that, a few degrees before this point is reached, the temperature, though not fatal, should yet be harmful and produce a diminution in the amount of assimilation of which the leaf is capable, such diminution being greater the nearer the temperature to the temperature of death.

From a consideration of the numbers obtained in these experiments we should expect that it would be impossible to produce greater assimilation at 41° C., 43° C., but that under the influence of increased light the leaf should be capable of greater assimilation at temperatures between 25° C. and 38° C.; possibly at these temperatures themselves.

It will be as well to consider here the evidence that when employing two ordinary

Table I



incandescent burners as the source of light, the temperature of the leaf was not appreciably different from that of the bath. In the experiment at 11° C., the light was changed considerably in intensity without producing an alteration in the amount of the assimilation. If the light had raised the temperature of the leaf above that of the bath, the substitution of the less intense light must have lowered the leaf-temperature, and therefore made a difference in the possible amount of assimilation. This is good evidence that at 11° C. the leaf was not appreciably affected in temperature by this light and thus, if this were so at 11° C. when there was excess of light, it would also be so at the other temperature when the light was obviously insufficient.

(iii) *Experiments with High Intensities of Light.*

(a) *Preliminary Experiments.*

The results of the first of experiments performed with the Keith high-pressure burners are given in Tables VIII., IX., and X. Those in VIII. and IX. were carried out before the necessity of determining the internal temperature was realised. In these, and all succeeding experiments, it was necessary to supply a greatly increased amount of CO₂, in order that the results might in no way depend on the percentage of CO₂. In consequence, in order not to risk incomplete absorption in the Pottendorfer tubes, each was used for only 1 hour. Since the amounts of CO₂ to be determined were so large, there was no objection to using this shorter time interval. The true temperature of the leaf was not determined in these preliminary experiments, but must have been several degrees higher than that of the bath. This

is clearly shown by the fact that the value obtained for the assimilation when the bath was at 11°C ., is considerably higher than the amount shown in Section IV. (ii) to be maximal for that temperature.

The highest value for the assimilation we have found occurs in Experiment L. I believe that this is also the highest value recorded by any observer.

Table IX. gives the results of some preliminary experiments illustrating the rise in the temperature of the leaf produced by the direct radiation of an intense light. The temperature of the bath was 32°C ., a temperature at which a leaf can live for over a week without being outwardly affected. Under the influence of the intense light, however, large brown patches appeared, the leaf became flaccid, and lost very rapidly a third of its weight, its behaviour thus resembling that of a leaf which has been exposed to a temperature of 43°C . or 45°C . The values obtained for the assimilation are very low, and it will be remembered that in Section IV. (ii), the experiments with weak light, it was shown that there was rapid decrease in the assimilation at temperatures above 38°C .

An interesting experiment is recorded in Table X. The assimilation of one leaf was determined under varying intensities of light, the temperature of the bath being 11°C . The arrangement of three Keith high-pressure burners was employed, the intensity of the light being altered by increasing their distance from the leaf. Now had the leaf been at the same temperature as the bath, the highest intensity of the light should have given a value of the assimilation of '0066 gramme per hour approximately (according to Experiment XXXVII.), and no change should have been produced in this value until the light had been decreased to an intensity less than fourfold. Table X. shows that the actual results obtained were very different. The initial value of the assimilation was '0100 gramme per hour, and this decreased continuously. There was no indication of the existence of a maximal amount of assimilation. This is entirely due to the heating effect of the excess light. Each additional increase in the intensity of the light produced a further increase in the temperature of the leaf, and, therefore, permitted of an increase in the assimilation.*

These preliminary experiments afford conclusive evidence of the necessity of determining the internal temperature of the leaf during an assimilation experiment when using an intense light.

(b) *Method of Determining the Internal Temperature of a Leaf.*

The temperature was determined by measuring, by means of a galvanometer, the electromotive force produced by the difference in temperature of two thermo-elements, one being embedded in the tissue of the leaf and the other being kept at a known temperature.

* The probable temperatures were determined later electrically by placing another leaf in exactly the same positions. The results obtained will be found in Column 3 of Table X.

The galvanometer used was an ordinary Thomson mirror-galvanometer, about $\frac{1}{2}$ ohm in resistance. The deflections were read directly on the scale; no refinements, such as the use of a telescope, were employed. A deflection of about five scale-divisions corresponded to a change in temperature of one degree, and it was possible to read to half a division. The error in the temperature determinations was found to be about half a degree, so that the direct method of reading the deflections was well within the limits of accuracy imposed by other conditions.

The thermo-elements were composed of copper and constantan (an alloy of copper and nickel), as these give a relatively large electromotive force.

It was a matter of some difficulty to obtain a thermo-element sufficiently small to be inserted in the leaf. Satisfactory junctions were made for this purpose by the Camb. Sci. Inst. Co. Extremely fine wires of copper and constantan were joined so skilfully that the junction was not much thicker than the wires themselves, and this was afterwards filed down until the place of union was hardly distinguishable from the general substance of the wire. Naturally they were in consequence extremely fragile and difficult of manipulation,* for the wires themselves were so fine that they frequently broke if sharply bent. It was a difficult and tedious task to embed these elements in the lamina, and since experiment showed that the temperature registered was the same in both positions, they were always embedded in the tissue of the midrib. The latter was first pierced, parallel to the blade of the leaf, with a very fine needle, the end of the wire was pushed through this tiny canal by forceps, and the thermo-element was then drawn through until the junction was just buried in the midrib. To avoid any chemical action of the leaf-juices on the wires, and to prevent any possible short circuiting through the tissue of the leaf, the element was insulated by coating it with a very thin layer of varnish, flexible enough not to crack when bent.

A specialised form of leaf chamber was necessary which should permit the wires from the thermo-element to pass out for connection with the galvanometer. An adaptation was made in the back of the chamber ordinarily used. A cork bored with two holes was fitted into the centre of the glass. Two narrow glass tubes, U-shaped, passed through the cork and formed an appendage to the chamber. A small quantity of mercury was poured into the bend of each tube,† and wires of copper and constantan, similar to those employed in making the thermo-element, were pushed down the long free limbs of these tubes until they dipped into the mercury, when they were fixed in position by inserting a small wooden peg into the open end of the glass tube. The free ends of these wires, i.e., those not in the

* On account of their fragile nature it was necessary to use a new thermo-element for almost every determination. The diameter of the wires was only .087 millim.

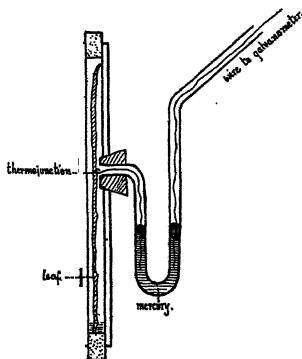
† In spite of the small surface of mercury exposed, it was found that enough vapour was given off to seriously affect the assimilating leaf, turning it black. This was due to the marked poisonous action of mercury vapour on green plants when assimilating, and was effectually prevented by covering the surface of the mercury with a film of water; see BOUSSINGAULT (18).

mercury, served to make connection with the other element and with the galvanometer. The wires of the thermo-element, projecting from the leaf, were inserted into the short limbs of the U-tubes and were carefully pushed down by forceps until their ends also dipped into mercury, and were, therefore, in electrical connection with the wires already in the U-tubes.

The glass, with its appendage of U-tubes and with the leaf in position, was then waxed on to the chamber.

The following diagram of the chamber and one of the two U-tubes may, perhaps, make this arrangement clear.

This arrangement of the U-tubes with the mercury and the accessory wires was, of course, permanent. The setting up, required afresh for each experiment, consisted in threading an element through the leaf, inserting the ends of these wires in the tubes, and waxing the glass on to the leaf chamber, the most difficult part of the manipulation being the insertion of the ends of the thermo-element into the U-tubes without breaking the wires. Sufficient water was afterwards introduced through the air-inlet tube to cover the petiole of the leaf. The leaf chamber was then placed in position in the bath and connected with the CO_2 current and absorption apparatus.



Section through leaf and chamber and one of the two U-tubes.

Another thermo-element at a known temperature was, of course, necessary. This second thermo-element (which, for convenience of reference, I will term the control-element) was threaded along an indiarubber tube of very narrow bore to insulate it, and to prevent contact with the water. This rubber tube was then tied to a thermometer, so that the thermo-electric junction was just against the bulb. This stood in a vessel of water which could be brought to any desired temperature.

Very thick copper wire was employed for making the connections with the galvanometer, since the latter had to be placed in the next room to avoid disturbances due to the working of the water-motor supplying the light. It was found most expedient to connect these wires to those from the thermo-elements by means of mercury cups. These were all fixed on the edge of the bath to insure equality of temperature, lest any slight difference in purity between the thin and the thick copper wires might cause them to act as secondary thermo-elements. The constantan wire from the thermo-element in the leaf was connected with that from the control thermo-element by another mercury cup.

The temperature was not read directly by means of the galvanometer, but a zero

method was employed, *i.e.*, the temperature of the water round the control element was adjusted until there was no deflection. The temperature of the control thermometer then indicated the real temperature of the leaf. This method worked very satisfactorily, and it was easy to determine the temperature to within half a degree, an accuracy quite sufficient for the purpose.

(c) *Final Experiments.*

In Section III. experimental evidence was given showing that from -6°C. to $+11^{\circ}\text{C.}$, if sufficient light be provided, the assimilation increases with the temperature. In the present section it has been possible to prove, by determining the internal temperature of the leaf, that this relation is maintained to within a few degrees of the death-point of the leaf.

The general course of procedure was as follows:—Immediately after setting up, the leaf was exposed to the light, and after a preliminary of $1\frac{1}{2}$ hours, four estimations (1 hour each) of the assimilation were made. The internal temperature of the leaf was determined at the beginning and end at least of every reading. A current of air free from CO_2 was then substituted and the light removed. The leaf was now at the temperature of the bath, *i.e.*, several degrees below what it had been during exposure to the light, the temperature at which its respiration was required. Hot water was therefore poured into the bath until the bath-thermometer registered the same temperature as that found, by means of the thermo-element, for the leaf during assimilation. The bath-thermostat was readjusted and the bath darkened by a black cloth. After an interval of 2 hours, two or three 2-hour estimations were made of the respiration.

All the leaves used in this series were picked on the same day and kept in beakers in the manner described on p. 61.*

As we can no longer (on account of the rise in internal temperature produced in the leaf) prove that the assimilation is maximal by merely showing that an increase in light produces no effect, it will be necessary to discuss each experiment separately and to prove that in each individual case we are dealing with the maximal assimilation for the particular temperature.

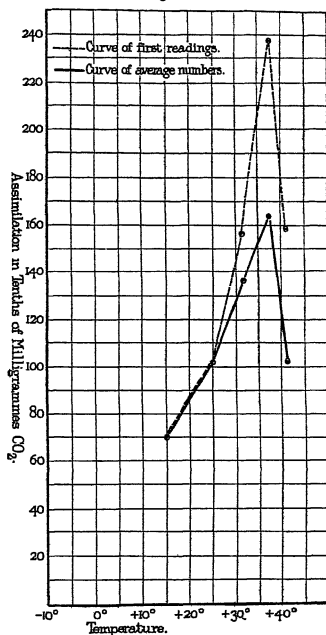
The true temperature of the leaf is dependent partly on the temperature of the bath and partly on the intensity of the light. The greater the intensity of the light, the greater must be the difference between the temperature of the bath and the required temperature of the leaf.

Experiment LVI., 15°C. —The intensity of the light employed in this experiment was thirteen times unit intensity. The lowest temperature of the bath attainable was 11°C. (that of running water at that time of year), and, in consequence, the

* Since the leaves were just passing through a period of seasonal change (see p. 82) it was necessary, in order to insure uniformity of condition, to gather them all on the same day.

excess of light must not be great enough to raise the temperature of the leaf more than 4°C .

Fig. 5.



Under these conditions the leaf decomposed $0.070\frac{1}{2}$ gramme CO_2 per hour. Now it will be seen that in Experiment XXXVII. at 11°C . the intensity of the light used was eight times unit intensity, *i.e.*, a little more than half that available in this experiment. The amount of CO_2 decomposed per hour was, however, 0.072 gramme, and therefore this leaf at 15°C . must be exposed to nearly twice the light necessary for the assimilation which it has actually performed. There is no doubt, therefore, that $0.070\frac{1}{2}$ is the present true value of the maximal assimilation at 15°C ., the excess of light being used up in raising the temperature of the leaf from 11 – 15°C ., and in maintaining it at this temperature despite the loss by radiation to the bath.

It will be seen from the *précis* of this experiment given in Table XI. that only one long estimation of the respiration was made. The amount of CO_2 produced by a single leaf at 15°C . is so small that it is impossible to make 2-hour estimations with sufficient accuracy.

It should also be noticed that there is very good agreement between the successive

numbers obtained for the value of the apparent assimilation, proving that at this temperature there is no decrease in the assimilation due to prolonged exposure.

Experiment LVII., 23°·7 C.—The temperature of the bath was again 11° C., but the intensity of the light was twenty-six times unit intensity.

The value of the assimilation is ·0101 gramme per hour, less than the amount (·0128) induced in Experiment XXXVIII., Table VII., by one-third of the light. Here again, therefore, there is no doubt that we are dealing with the maximal assimilation for 23°·7 C.

At this temperature, also, there is no decrease in the apparent assimilation during the course of the experiment.

Experiment LVIII., 30°·5 C.—To obtain this temperature an intensity of light equivalent to L.In. = 45 and a bath temperature of 13°·5 C. were employed. To show that here the assimilation is maximal we must turn to the experiments in Table VIII. There the leaf chamber was in exactly the same position in the bath, the same burners were used, but were placed several millimetres further away. The value of the assimilation induced by this less intense light was, however, ·0249 gramme per hour, showing that the light employed in the present experiment would have been sufficient to produce more assimilation than this, and therefore the value of ·0186 gramme per hour must represent the maximal assimilation at this temperature.

Here the value found for the apparent assimilation decreases during the course of the experiment, the most rapid fall taking place between the first two readings. This is due mainly to the diminution in the assimilatory activity of the leaf when the absolute amount of assimilation is high.

Experiment LIX., 37°·5 C.—The intensity of the light used was the same as in the previous experiment, and, therefore, the same arguments apply, since the assimilation, even in the first reading, is not as great as ·0249 gramme per hour. Thus we have here again maximal assimilation.

In this experiment the sudden and large changes in the value of the apparent assimilation are very striking. The value obtained in the first reading is very large, that in the second is only two-thirds of the first. This fall continues during the third and fourth readings, but is less rapid. The average value obtained from the four readings, however, is quite in harmony, as regards its position in the curve, with the results obtained at the other temperatures.

Experiment LX., 40°·5 C.—The intensity of the light used was the same as in the last experiment, and since the assimilation is so much less, it must *a fortiori* represent the maximal value.

Here the high temperature exerts a decidedly injurious effect. Although the temperature is only 3° above that of the previous experiment, the value of the assimilation in the first readings has fallen from ·0237 to ·0149.

The temperature (37°·5 C.) forms the turning point in the curve. Two influences are

at work, firstly, the effect of the temperature hitherto experienced, *i.e.*, that of raising the amount of assimilation of which the leaf is capable, and, secondly, the injurious effect of a high temperature of which the tendency is to depress the assimilation. (See experiments at 41° C. and 43° C. in Table VII.)

This, the highest point of the curve, is, therefore, a position of unstable equilibrium. At the beginning of the experiment the leaf is under the optimal conditions for assimilation, and we get the greatest possible value for the maximal assimilation. Immediately after this effort, however, the injurious effect of the temperature gains the ascendancy, and we have the initial rapid fall* which subsequently becomes more gradual. That the optimal temperature for the assimilation must produce effects of this nature is obvious, for if the effect of rise in temperature is to become of a completely opposite character, there must be some point or region where both these tendencies make themselves felt.

At 15° C. and 23°·7 C. there was no change in the value of the assimilation during the experiment; at 30°·5 C. the first reading was slightly, although not markedly, higher than the others, and we have the fullest expression of this change at 37°·5 C. The question now arises what value is to be taken as representing the maximal assimilation at these temperatures? Two different curves (although both of the same type) can be obtained by using either the numbers representing the average of the same number of estimations in each case or the numbers of the first readings only. Either curve gives the true conception of the relation between temperature and assimilation, namely, that when the other conditions are optimal, it is only rise in temperature which renders rise in assimilation possible.

The curve more truly representing the pure effect of the rise in temperature on the assimilation will, however, be that derived from the numbers obtained in the first readings only, for these are the most nearly unaffected by prolonged exposure. At 15° C. and 23°·7 C. the value derived from the first reading is practically the same as the average value; at 30°·5 C. it is ·0157 instead of ·0136; at 37°·5 C., ·0237 instead of ·0163.

The upper curve in fig. 5 is based on these first readings, and must be regarded as the most correct expression of the effect of temperature on assimilation. It much resembles the curve of the effect of temperature upon respiration.

The lower curve, that formed by using the average numbers, is another expression of the same relation, but in this case the depressing influence of comparative high temperatures is also taken into account. It is, therefore, of a more composite nature, and expresses the average effect on a leaf of 6 hours' assimilation at these various temperatures. It is quite evident that by varying the number of assimilation readings entering into the average we could obtain a whole series of curves of the same type.

* There is ample evidence that the sudden decrease between the amounts in the first two readings is not in any way due to a choking effect produced by the large amount of carbo-hydrate formed during the first hour, but is a simple temperature effect.

We might even get quite a different temperature to represent the optimal temperature for assimilation, according to the length of time which was allowed to elapse before readings were taken.

Section V.—SUMMARY AND CONCLUSIONS.

i. *Recapitulation.*

Let us now recall the evidence at our disposal on the question of the relation between temperature and assimilation. In the first place we have the series of curves in figs. 2 and 3 (Section III.). These curves are all of the same type; they show an initial rising portion, changing abruptly into a level line. The greater the intensity of the light, the higher up in the temperature-scale does this change in form occur. The steeply rising portion of the curve is an expression of the fact that, at the temperatures concerned, the intensity of the light is sufficient for maximal assimilation, the long level portion merely gives the amount of CO_2 the light employed was capable of decomposing. From these curves we have obtained the maximal assimilation from -6°C. , where the assimilation begins, to $+11^\circ \text{C.}$

We next consider the evidence given by the curve in fig. 4, embodying the results of the experiments in Section IV. (ii.). This is again a curve of the same type as the earlier ones and shows the insufficiency of the eightfold light for maximal assimilation in the middle part of the temperature range. The decided fall at the very high temperatures was a new feature. As each number employed in the curve is the mean of two separate experiments, this steep fall is well established.

It is of interest to compare with this curve the final curve given by KREUSLER (1) for the relation between the assimilation and the temperature. The forms are almost identical, thus clearly showing that the curve he obtained was not the true one but was conditioned as to its middle part by insufficiency of the light reaching the leaves.

We now come to the final curve given in fig. 5 (Section IV. (iii.), c.), comprising the range of temperatures from $15-41^\circ \text{C.}$ Here we have full evidence that at every point the assimilation is maximal and the curve obtained rises more steeply the higher the temperature. Above 38°C. , however, we have the steep fall, the existence of which was shown in fig. 4.

ii. *Seasonal Variation in the Activity of Leaves.*

On comparing the amounts of assimilation represented in figs. 4 and 5 an apparent anomaly becomes obvious. The actual amounts in fig. 5 are very much smaller than those in fig. 4, although the latter were obviously not maximal at that time. On the other hand, there was conclusive evidence, Section IV. (iii.), c, that in these later experiments the low values represented the maximal assimilation. The explanation is to be found in a seasonal change in the condition of the leaves.

Fig. 4 is based on experiments performed during the winter months, for the main part in February, 1903; fig. 5 on experiments carried out in April, 1903. A very short time before the latter experiments, the weather had become decidedly warmer, and a very marked change had taken place in the vegetation generally. This passage of winter into spring is connected with a marked *decrease* in the assimilatory activity of the leaves; it appears as if at the change of weather they suddenly became sluggish.

The same phenomenon may be noticed on comparing the amounts of assimilation obtained with unit intensity of light in fig. 2 (February, 1902) and fig. 3 (April, 1902). Evidently in April the leaf does not exert its full capability of assimilation, for only '0020 gramme CO_2 is decomposed under the influence of an intensity of light which in February was sufficient for the breaking up of '0028 gramme CO_2 .

In what way this reduction in the activity is brought about is not yet determined and it is by no means clear what the immediate cause may be. It appears as if in the sluggish condition of the leaves more light were necessary to do the same amount of work.

Frequent experiments with leaves off the same bush must be made at various times of year to throw more light on this seasonal change, and the investigation is in hand. It may be of interest to mention that both in 1901 and 1902 I obtained a low value for the assimilation from October to the middle of December, when the weather was comparatively mild, a high value from the middle of January to March, which was the coldest part of the year, and a low value again in April. The young leaves appear in May, develop slowly during the summer and do not reach a mature state until September.

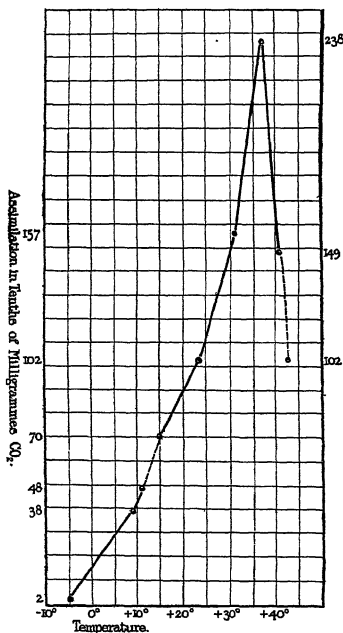
We have shown that these phenomena do not affect the general form of the curve obtained, but only the absolute amounts representing the maximal assimilation at the various temperatures. The maximal assimilation of a leaf at 30°C . might be '0240 gramme in February, and in April be '0136 gramme, but a reduction in the temperature to 11°C . would cause the same proportionate decrease in both cases.

iii. *Synthesis of Complete Curve.*

In synthesising from the figures, given before, the complete curve showing the relation between temperature and assimilation, this effect of the seasonal change on the value of the assimilation must be borne in mind. The experiments represented in figs. 3 and 5 were all carried out in April, during the period of decreased activity. From fig. 3 we obtain the value for the maximal assimilation at all temperatures from -6°C . to 11°C .; from fig. 5 for all temperatures from 15°C . to $40\cdot5^\circ \text{C}$. These values are placed together in the accompanying diagram (fig. 6), and it will be seen that fig. 5 forms an almost exact continuation of fig. 3. From fig. 4 we know that after 40°C . the assimilation rapidly decreases up to 43°C ., we may, therefore,

continue the steep decrease in fig. 6. This gives the complete curve from -6°C . to $+43^{\circ}\text{C}$. Therefore, under optimal conditions of light and supply of CO_2 , the

Fig. 6.



assimilation rises rapidly with rise of temperature until within a few degrees of the temperature fatal to the leaf.

We see then that for every temperature there is a certain intensity of light beyond which further increase in the illumination produces no effect on the assimilation, except indirectly by increasing the internal temperature of the leaf. The influence of the intensity of illumination and of the percentage of CO_2 , are therefore of the same secondary nature, and can only affect the assimilation by reducing it if they are below the required optimal amount for the particular temperature.

iv. Conclusions.

- (1) Corresponding to each temperature there is a certain definite amount of assimilation which may be termed the "maximal assimilation for that temperature,"

this cannot be exceeded, and will not be reached unless both light and CO_2 supply are adequate.

(2) These maximal amounts increase rapidly with increasing temperature, forming a curve which is convex to the temperature abscissa and resembles the curve of the relation between temperature and respiration.

(3) This series of maxima begins to decrease suddenly some degrees below the fatal temperature (*i.e.*, temperature sustainable only for a few hours).

(4) At high temperatures an individual leaf can only maintain a maximal assimilation for a short time. The higher the temperature the sooner the decline begins and the steeper its slope. It follows that a curve of the values of the assimilation at different temperatures in the first hour is not the same as that formed from the values during the second hour, &c.

(5) The assimilatory activity of a mature leaf is not always the same, but varies considerably with the time of year.

I wish finally to acknowledge my indebtedness to the Royal Society for a grant towards the cost of the apparatus employed.

BIBLIOGRAPHY.

1. KREUSLER. "Beobachtungen über die Kohlensäure-Aufnahme und Ausgabe der Pflanzen," 'Landwirthschaftliche Jahrbücher,' 1887, p. 711.
2. PFEFFER. 'The Physiology of Plants,' vol. 1, Section 58.
3. BOUSSINGAULT. 'Agronomie,' 1868, vol. 4, p. 312.
4. SAPOSCHNIKOFF. "Über die Grenzen der Anhäufung der Kohlenhydrate," 'Ber. d. D. Bot. Ges.,' 1890, p. 298.
 ——— "Beitrag zur Kenntniss der Grenzen der Anhäufung von Kohlenhydraten in den Blättern," 'Ber. d. D. Bot. Ges.,' 1893, p. 391.
 "Eiweisstoffe und Kohlenhydrate der Grünen Blätter als Assimilationsproducte," 'Bot. Centralblatt,' 1895, vol. 63, p. 246.
5. BORODIN. "Untersuchungen über die Pflanzenathmung," 'Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg,' 7 série, vol. 28, p. 4.
6. CLOËZ and GRATIOT. "Recherches Expérimentales sur la Végétation des Plantes submergées," 'Ann. Sci. Nat.,' S. III, vol. 1, 32, 1851, p. 42.
7. BOUSSINGAULT. 'Agronomie,' vol. 5, p. 16.
8. FRANCOPERT. "Recherches sur la Respiration des Végétaux," 'Comptes Rendus,' 1864, vol. 58, p. 334.
9. BOEHM. 'Sitzungsbericht d. Wien. Akad,' 1873, vol. 1, p. 52.

10. HEINRICH. "Beiträge zur Kenntniss des Temperatur- und Licht-einflusses auf die Sauerstoffausscheidung bei Wasserpflanzen," 'Landwirthschaft. Versuchstationen,' vol. 13, 1871.
11. PFEFFER. "Die Wirkung farbigen Lichtes auf die Zersetzung der Kohlensäure in Pflanzen," 'Arb. d. Bot. Inst. zu Würzburg,' 1874, vol. 1, p. 52.
12. REINKE. "Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoffausscheidung der Pflanzen," 'Bot. Zeit.,' 1884, p. 24.
13. PRJANISCHNIKOW. "Wirkung des Lichtes und der Wärme auf das Ergrünen und auf den Gas-austausch der Pflanzen," Warschau, 1876. 'Botanisches Jahresbericht,' 1876.
14. VAN TIEGHEM. 'Traité de Botanique,' vol. 1, p. 179.
15. JUMELLE. "Recherches physiologiques sur les Lichens," 'Revue Générale de Botanique,' 1892, p. 303.
16. EWART. "Assimilatory Inhibition in Plants," 'Linn. Soc. Journal,' Botany, 1895, vol. 31, p. 389.
17. REINKE. "Untersuchungen über die Einwirkung des Lichtes auf die Sauerstoff-ausscheidung der Pflanzen," 'Bot. Zeit.,' 1883, p. 713.
18. BOUSSINGAULT. 'Agronomie,' 1868, vol. 4, p. 343.

VEGETABLE ASSIMILATION AND RESPIRATION.

TABLES OF EXPERIMENTS.

- I. *Weight and Area*.—Where two weights are given in this column the first number denotes the weight before the experiment, and the second after. The mean is taken as the true weight of the leaf. When only one is given it is the weight before the experiment.

The assimilation has always been calculated per area, the respiration per weight (see p. 64).

- II. *Respiration*.—The values in all the tables, except VII. and XI., are obtained from the data of Table I. (last column) either directly or by interpolation (see the curve of fig. 2).

- III. *Apparent Assimilation*.—The apparent assimilation is the amount of CO_2 removed by the leaf in the light from the air-current passing over it, and is measured by the excess of the CO_2 shown by the control tubes over that of the tubes from the chamber. This amount, together with the respiratory CO_2 which the leaf may be considered at that moment as producing, indicates the amount of real assimilation which the leaf is performing.

- IV. *Estimations of the CO_2* .—All the amounts are expressed in grammes of CO_2 .

- V. *Aspirator Rate*.—The rate of flow of the aspirators was always about 800 cub. centims. per hour.

- VI. *Percentage of CO_2* .—The percentage of CO_2 employed varied between .8 and 2.8, and can easily be obtained in any particular case.

$$\text{e.g., per cent. of } \text{CO}_2 \text{ by volume} = \frac{\text{weight of } \text{CO}_2 \text{ in control tube per hour} \times 500}{8},$$

since the rate of the current is 800 cub. centims. per hour and 500 cub. centims. CO_2 weighs approximately 1 gramme.

- VII. *Intensity of Light*.—The light obtained from a single ordinary incandescent mantle when the front of the mantle is 130 millims. from the leaf is termed light of unit intensity, expressed thus—L.In. = 1.

All other intensities of light are measured in terms of this unit—L.In. = 4, &c.

TAB. I. Results of experiments at 3° C.

Date.	Temperature.	Number of leaves.	Weight before experiment.	Weight after experiment.	Total amount CO ₂ for each 2 hours' reading.					Calculated amount CO ₂ per hour per 2 grammes.					Mean value CO ₂ per hour per 2 grammes.
					1	2	3	4	5	1	2	3	4	5	
February 4-6, 1902. 2.55 P.M.-2.55 A.M.	5°-8 C.	11	18.80	18.55	.0019	.0019	.0019	.0020 $\frac{1}{2}$	.0019 $\frac{1}{2}$	.0001	.0001	.0001	.0001	.0001	.0001
January 31-February 1, 1902 5.40 P.M.-6.20 A.M.	14°-2 C.	8	12.67	12.75	.0081	.0081	.0083	.0082	.0082	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$
February 5-6, 1902. 5.30 P.M.-8.30 A.M.	14°-8 C.	8	12.75	12.80	.0052 $\frac{1}{2}$	.0052 $\frac{1}{2}$	.0056	.0056	.0056 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0002 $\frac{1}{2}$	.0003	.0003	.0003	.0003
January 30-31, 1902. 8.20 P.M.-8.30 A.M.	18°-2 C.	7	10.10	10.15	.0052	.0049 $\frac{1}{2}$	.0045 $\frac{1}{2}$	—	.0046	.0005	.0005	.0004 $\frac{1}{2}$	—	.0004 $\frac{1}{2}$	.0004 $\frac{1}{2}$
February 9-7, 1902. 8.30 P.M.-8.30 A.M.	18°-1 C.	7	11.77	11.82	.0050 $\frac{1}{2}$	.0050	.0049	.0048	.0047 $\frac{1}{2}$	.0004 $\frac{1}{2}$	.0004	.0004	.0004	.0004	.0004
January 26-28, 1902. 8 P.M.-8 A.M.	25°-7 C.	5	7.68	7.72	.0056 $\frac{1}{2}$	.0052	.0045 $\frac{1}{2}$	.0045 $\frac{1}{2}$	.0041	.0007 $\frac{1}{2}$	.0007	.0006	.0006	.0005 $\frac{1}{2}$	.0006
January 24, 1902. 11.20 A.M.-11 P.M.	28°-2 C.	4	6.25	6.40	.0054 $\frac{1}{2}$	.0054 $\frac{1}{2}$	.0053	.0050 $\frac{1}{2}$	.0045	.0009	.0009	.0008 $\frac{1}{2}$	.0008	.0007	.0008 $\frac{1}{2}$
January 23, 1902. 10.30 A.M.-10.30 P.M.	35°-0 C., went up to 35°-8 C. at end of experiment.	2	8.35	8.37	.0046 $\frac{1}{2}$	.0047	.0048	.0048	.0051	.0014	.0014	.0014	.0014	.0015	.0014
February 9-10, 1902. 3 P.M.-3 A.M.	38°-1 C.	2	8.05	—	.0046 $\frac{1}{2}$	.0042	.0042 $\frac{1}{2}$	.0051	.0056 $\frac{1}{2}$	—	.0014	.0014	.0013 $\frac{1}{2}$	.0013 $\frac{1}{2}$	.0013 $\frac{1}{2}$

TABLE II.

Assimilation experiments at medium temperatures with unit intensity of light. Single ordinary incandescent burner at low gas pressure (1 inch water). One mantle used throughout.

Weight and area.	Temperature.		Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference	Respiration for leaf per 2 hours. [Calculated from Table I.]	Real assimilation for leaf for 2 hours.	Real assimilation calculated for 50 sq. cms. and 1 hour.
	Time.	Temp.							

Experiment V.—January 29, 1902. 14°·4 C.

1st weight, 1·60 grammes.	P.M.	14°·2	1.0 - 2.45 P.M.	Preliminary	Preliminary	—	—	—	—
2nd weight, 1·62 grammes.	3.0 "	14°·3	2.45- 4.45 "	·031½	·0280½	·0051	·0004	·0055	·0027
Area, 51·6 sq. centims.	4.15 "	13°·8	4.45- 5.15 "	·0356	·0280	·0050	·0004	·0054	·0026½
	7.10 "	14°·6	5.45- 8.45 "	·031½	·0280	·0051½	·0004	·0055½	·0027
	8.20 "	14°·5	8.45-10.45 "	·0334	·0273	·0061	·0004	·0065	·002½
	10.45 "	14°·0	10.45-12.45 A.M.	·035½	·0275½	·0056	·0004	·0060	·0025

Experiment VI. 18°·0 C.

1st weight, 1·60 grammes.	11.0 A.M.	18°·0	10.15-11.45 A.M.	Preliminary	Preliminary	—	—	—	—
2nd weight, 1·60 grammes.	12.45 P.M.	18°·0	11.45- 1.45 P.M.	·0323	·0281	·0042	·0007	·0049	·0027
Area, 44·8 sq. centims.	3.40 "	17°·8	1.45- 3.45 "	·0323½	·0280½	·0043	·0007	·0050	·0027½
	4.40 "	18°·1	3.45- 5.45 "	·0322½	·0280	·0042½	·0007	·0049½	·0027
	6.45 "	18°·2	5.45- 7.45 "	·0330½	·0288½	·0044	·0007	·0051	·0028
	8.20 "	18°·2							

Experiment VII.—January 25, 1902. 25°·7 C.

1st weight, 1·68 grammes.	11.0 A.M.	25°·6	10.15-11.20 A.M.	Preliminary	Preliminary	—	—	—	—
2nd weight, 1·64 grammes.	12.20 noon	25°·6	11.20- 1.20 P.M.	·0327	·0278½	·0048½	·0011	·0059½	·0029½
Area, 50·0 sq. centims.	1.0 P.M.	25°·6	1.20- 3.20 "	·0323½	·0273	·0050½	·0011	·0061½	·0030½
	4.0 "	25°·6	3.20- 5.20 "	·0321	·0268	·0053	·0011	·0064	·0032
	7.30 "	25°·8	5.20- 7.20 "	·0321	·0270	·0051	·0011	·0062	·0031

Experiment VIII.—January 23, 1902. 29°·1 C.

1st weight, 1·88 grammes.	10.40 A.M.	29°·0	10.30-12.20 P.M.	Preliminary	Preliminary	—	—	—	—
2nd weight, 1·86 grammes.	12.45 P.M.	29°·1	12.20- 2.20 "	·0338½	·0297½	·0036	·0012	·0048	·0028
Area, 43·0 sq. centims.	2.30 "	29°·2	2.20- 4.20 "	·0359	·0294	·0035	·0012	·0047	·0027½
	4.50 "	29°·2	4.20- 6.20 "	·0333	·0292½	·0040½	·0012	·0052½	·0030½
	8.0 "	29°·2	6.20- 8.20 "	·0323½	·0288½	·0035	·0012	·0047	·0027½

Experiment IX.—February 1, 1902. 33°·1 C.

1st weight, 1·45 grammes.	12.40 P.M.	33°·0	10.45-12.45 P.M.	Preliminary	Preliminary	—	—	—	—
Area, 33·5 sq. centims.	1.0 "	33°·0	12.45- 2.45 "	·0317½	·0288	·0029½	·0020	·0049½	·0028½
	3.20 "	33°·2	2.45- 4.45 "	·0324½	·0295	·0029½	·0020	·0049½	·0028½
Subsequent readings spoilt.									

TABLE III.

Further assimilation experiments at medium temperatures with unit intensity of light. Single incandescent burner at low gas pressure (1 inch water). One mantle employed throughout.

Weight and area.	Temperature.		Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Respiration for leaf per 2 hours. [Calculated from Table I.]	Real assimilation for leaf for 2 hours.	Real assimilation calculated for 50 sq. cms. and 1 hour.
	Time.	Temp.							
Experiment X.—February 8, 1902. 5°·5 C.									
Weight, 1·60 grammes. Area, 41·5 sq. centims.	10.45 A.M.	5°5	10.45–11.50 A.M.	Preliminary	Preliminary	—	—	—	—
	12 noon	5·5	11.15–1.15 P.M.	·0304½	·0258	·0046½	·0001½	·0048	·0029
	2.0 P.M.	5·6	1.15–3.15 "	·0316½	·0270	·0046½	·0001½	·0048	·0029
			3.15–6.15 "	·0313	·0269	·0044	·0001½	·0046½	·0027½
Experiment XI.—February 6, 1902. 10°·2 C.									
Weight, 1·70 grammes. Area, 49·8 sq. centims.	10.40 A.M.	10°0	10.20–11.50 A.M.	Preliminary	Preliminary	—	—	—	—
	2.10 P.M.	10·1	11.50–1.50 P.M.	·0254	·0203½	·0045½	·0002½	·0048	·0024*
	4.0 "	10·2	1.50–3.50 "	·0297½	·0241	·0056½	·0002½	·0059	·0028½
	7.40 "	10·4	3.50–5.50 "	·0325	·0271½	·0053½	·0002½	·0056	·0028
Experiment XII.—February 7, 1902. 10°·1 C.									
Weight, 1·60 grammes. Area, 42·5 sq. centims.	10.0 A.M.	10°05	9.50–2.30 P.M.	Preliminary	Preliminary	—	—	—	—
	4.30 P.M.	10°05	2.30–4.30 "	·0325	·0279½	·0045½	·0002½	·0048	·0028
	5.5 "	10°05	4.30–6.30 "	·0327	·0291½	·0045½	·0002½	·0048	·0028
	8.40 "	10·1	6.30–8.30 "	·0334	·0288	·0046	·0002½	·0048½	·0028½
Experiment XIII.—February 5, 1902. 14°·2 C.									
1st weight, 1·45 grammes. 2nd weight, 1·42 grammes. Area, 43·2 sq. centims.	10.30 A.M.	14°2	10.10–11.50 A.M.	Preliminary	Preliminary	—	—	—	—
	12 noon	14·2	11.50–1.50 P.M.	·039½	·0283½	·0056	·0004	·0060	·0035
	2.45 P.M.	14·2	1.50–3.50 "	·0391½	·0276½	·0055	·0004	·0059	·0034½
	5.45 "	14·2	3.50–5.50 "	·0387	·0290½	·0056½	·0004	·0060½	·0035
Experiment XIV.—February 11, 1902. 14°·0 C.									
Weight, 1·60 grammes. Area, 45·3 sq. centims.	12 noon	14°05	12.0–2.0 P.M.	Preliminary	Preliminary	—	—	—	—
	2.45 P.M.	14°05	2.0–4.0 "	·0318½	·0274	·0044½	·0004	·0043½	·0027
	3.45 "	14°05	4.0–6.0 "	—	—	—	—	—	—
	6.35 "	13°9	6.0–8.0 "	·0318	·0276½	·0041½	·0004	·0045½	·0025
Experiment XV.—February 10, 1902. 25°·0 C.									
Weight, 1·55 grammes. Area, 45·7 sq. centims.	9.30 A.M.	25°0	9.30–11.30 A.M.	Preliminary	Preliminary	—	—	—	—
	12.30 P.M.	24°9	11.30–1.30 P.M.	·0316½	·0286½	·0050	·0010½	·0060½	·0032
	3.15 "	25°0	1.30–3.30 "	·0321	·0272½	·0048½	·0010½	·0059	·0031
	6.15 "	25·4	3.30–5.30 "	·0324	·0271	·0048	·0010½	·0063½	·0035

* At end of reading, a streak was noticed on window of bath, and to this the smallness of the amount is due.

TABLE IV.

Assimilation experiments at low temperatures with unit intensity of light. Single incandescent burner at low gas pressure (1 inch water). A new mantle for each experiment.

Weight and area.	Temperature.	Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Respiration for leaf per 2 hours. [Calculated from Table I.]	Real assimilation for leaf for 2 hours.	Real assimilation calculated for 50 sq. cms., and 1 hour.
Experiment XVI.—February 27, 1902. +1° C.								
1st weight, 1.68 grammes. 2nd weight, 1.63 grammes. Area, 43.8 sq. centims.	+ 0.9 to 1.1° C.	10.35-12.30 P.M.	Preliminary	Preliminary	—	—	—	—
		12.30-2.30 "	·0810	·0274	·0036	·0000½	·0039½	·0020½
		2.30-4.30 "	·0293	·0256½	·0035½	·0000½	·0037	·0021
		4.30-6.30 "	·0305½	·0272½	·0033	·0000½	·0033½	·0019
Experiment XVII.—March 2, 1902. +2° C.								
1st weight, 1.80 grammes. 2nd weight, 1.81 grammes. Area, 46.0 sq. centims.	+ 2° C. throughout.	10. 5-11.35 A.M.	Preliminary	Preliminary	—	—	—	—
		11.35-1.35 P.M.	·0270½	·0238	·0042½	·0000½	·0043	·0023½
		1.35-3.35 "	·0258	·0213½	·0044½	·0000½	·0015	·0024½
		3.35-5.35 "	·0269½	·0225	·0044½	·0000½	·0045	·0024½
Experiment XVIII.—February 13, 1902. +2° 4.								
1st weight, 1.82 grammes. 2nd weight, 1.82 grammes. Area, 49.0 sq. centims.	Generally, 2-4° C. Lowest temperature noted, 2° C. Highest, 2-5° C.	11.15-12.45 P.M.	Preliminary	Preliminary	—	—	—	—
		12.45-2.45 "	·0386½	·0285½	·0051	·0000½	·0051½	·0026
		2.45-4.45 "	·0380	·0278½	·0051½	·0000½	·0052	·0026½
		4.45-6.45 "	·0381	·0281½	·0049½	·0000½	·0050	·0025½
Experiment XIX.—March 7, 1902. 3°-6 C.								
1st weight, 1.40 grammes. 2nd weight, 1.45 grammes. Area, 45.8 sq. centims.	+ 3°-6 C. throughout.	10.20-11.50 A.M.	Preliminary	Preliminary	—	—	—	—
		11.50-1.50 P.M.	·0301	·0250	·0051	·0000½	·0051½	·0028½
		1.50-3.50 "	·0297½	·0246	·0051½	·0000½	·0052	·0028½
		3.50-5.50 "	·0290½	·0245	·0051½	·0000½	·0052	·0028½
	Temperature allowed to rise. 7.12 P.M., 8°-7 C. 10. 0 " 9°-3 C. 1. 0 A.M., 9°-2 C.	5.50-7.50 P.M.	·0302	·0253	·0049	·0002½	·0051½	·0028½
		7.50-9.50 "	·0294½	·0242	·0052½	·0003½	·0053	·0030
		9.50-11.50 "	·0284½	·0235	·0049½	·0002½	·0052	·0028½
Experiment XX.—March 4, 1902. 8°-8 C.								
1st weight, 1.68 grammes. 2nd weight, 1.76 grammes. Area, 48.2 sq. centims.	10.30 A.M., 8°-9 C. 11.15 " 8°-6 C. 2.30 P.M., 5°-9 C.	10.15-11.45 A.M. 11.45-1.45 P.M. 1.45-3.45 "	Preliminary ·0232 ·0218	Preliminary ·0176 ·0163	— ·0056 ·0055	·0002½ ·0002½ ·0002½	·0058½ ·0057½ ·0057½	·0030 ·0029½ ·0029½

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TABLE IV.—*continued.*

Weight and area.	Temperature.	Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Respiration* for leaf per 2 hours. [Calculated from Table I.]	Real assimilation for leaf for 2 hours.	Real assimilation calculated for 50 sq. cms. and 1 hour.
Experiment XXI.—March 1, 1902. - 6°·0 C.								
1st weight, 1·85 grammes. Area, 49·0 sq. centims.	About -6°·0 C. the whole time.	10.30- 1.0 P.M. 1.0 - 2.15 " 2.15- 4.15 " 4.15- 6.15 "	Pre-liminary "0303½ "0303½ "0311½	Preliminary "0299 "0304½	— "0004½ "0007	— — —	— "0001½ "0037	— "0002½ "0003½
Experiment XXII.—February 24, 1902. - 3°·6 C.								
1st weight, 1·78 grammes. Area, 48·7 sq. centims.	-3°·6 C. to -3°·6 C. with a few minutes at -0°·5 C. in first reading.	10.10-11.45 A.M. 11.45- 1.45 P.M. 1.45- 3.45 " 3.45- 5.45 "	Preliminary "0303½ "0300½ "0312½	Preliminary "0282 "0283 "0297	— "0021½ "0017½ "0015½	— — —	— "0021½ "0017½ "0015½	— "0011 "0000 "0003
Experiment XXIII.—February 20, 1902. - 1°·0 C.								
Weight, 1·66 grammes. Area, 44·0 sq. centims.	-2°·0 C. -1°·0 C. -1°·0 C.	10.45-12.15 P.M. 12.15- 2.15 " 2.15- 4.15 " 4.15- 6.15 "	Preliminary "0300½ "0307½ "0314½	Preliminary "0278½ "0280 "0288	— "0022 "0027½ "0026½	— — —	— "0022 "0027½ "0028½	— "0012½ "0015½ "0015
Experiment XXIV.—February 17, 1902. - 1°·5 C.								
1st weight, 1·67 grammes. 2nd weight, 1·67 grammes. Area, 45·7 sq. centims.	-1°·4 C. to 1°·6 C.	12.0 - 2.0 P.M. 2.0 - 4.0 " 4.0 - 6.0 " 6.0 - 8.0 "	Preliminary "0317 "0334 "0388	Preliminary "0289½ "0314 "0320	— "0017½ "0020 "0018	— — —	— "0017½ "0020 "0018	— "0010 "0011½ "0010

The respiration at temperatures below 0° C. amounts to less than the error of observation, and has therefore been neglected.

VEGETABLE ASSIMILATION AND RESPIRATION.

TABLE V.

Assimilation experiments at low and medium temperatures, with various low intensities of light.
Single incandescent burner at low gas pressure (1 inch water). New mantle each experiment.

Intensity of light.	Weight and area.	Temperature.	Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Apparent assimilation calculated for 50 sq. cms., and 1 hour.	Real assimilation* calculated for 50 sq. cms., and 1 hour.
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Experiment XXVIII.—May 3, 1902. 0°·4 C.

Unit.	1st weight, 1·68 grammes. 2nd weight, 1·70 grammes. Area, 40·4 sq. centims.	0°·4 C. to 0°·8 C. throughout.	9.30-11.0 A.M. 11. 0- 1.0 P.M. 1. 0- 3.0 "	Preliminary ·0295½ ·0805	Preliminary ·0289½ ·027½	— ·0026 ·0031	— ·0016 ·0019	— ·0016 ·0019
Twofold at 3 P.M.			3.10-4.0 P.M. 4. 0-6.0 " 6. 0-8.0 "	Preliminary ·0308 ·0800	Preliminary ·0272 ·0270	— ·0036 ·0030	— ·0022 ·0018½	— ·0022 ·0018½

Experiment XXIX.—April 12-13, 1902. 8°·8 C.

Unit.	1st weight, 1·80 grammes. Area, 41·8 sq. centims.	8°·8 C. throughout	12.30-2.0 P.M. 2. 0-4.0 " 4. 0-6.0 " 6. 0-8.0 "	Preliminary ·0818 ·0820 Spoilt	Preliminary ·0275½ ·0283 —	— ·0037½ ·0037	— ·0021 ·0020½	— ·0023 ·0022½
Twofold at 8 P.M.		8°·8 C. at 8 P.M.	8. 0- 9.40 P.M. 9.40-11.40 " 11.40- 1.40 A.M. 1.40- 3.40 " 3.40- 5.40 " 5.40- 7.40 "	Preliminary ·0831 ·0832½ ·0828 ·0831 ·0828	Preliminary ·026½ ·0266½ ·0263½ ·0266 ·0263	— ·0066½ ·0066 ·0064½ ·0065 ·0065	— ·0087 ·0037 ·0036 ·0036½ ·0038½	— ·0039 ·0039 ·0033 ·0038½ ·0038½
	2nd weight, 1·80 grammes.	9° C. at 9 A.M.						

Experiment XXX.—April 13-14, 1902. 9°·2 C.

Unit.	1st weight, 1·58 grammes. Area, 38·1 sq. centims.	5°·1 C. at 7 P.M.	6.50- 9.15 P.M. 9.15-11.15 " 11.15- 1.15 A.M. 1.15- 3.15 " 3.15- 5.15 " 5.15- 7.15 " 7.15- 9.40 "	Preliminary ·0831½ ·0834½ ·0830	Preliminary ·0248½ ·0250½ ·0246	— ·0033 ·0034 ·0034	— ·0018 ·0018½ ·0018½	— ·0020 ·0020½ ·0020½
Fourfold at 9.40 A.M.		9°·2 C. at 9 A.M.	9.40-10.50 A.M. 11.50-12.50 P.M. 12.50- 2.50 " 2.50- 4.50 " 4.50- 6.50 "	Preliminary ·0444 ·0444½ ·0441½ ·0440½	Preliminary ·0379½ ·0380 ·0380 ·037½	— ·0064½ ·0064½ ·0061½ ·0066½	— ·0035 ·0035 ·0033 ·0036	— ·0037 ·0037 ·0035 ·0033

* Respiration calculated from Table I.

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TABLE V.—*continued.*

Intensity of light.	Weight and area.	Temperature.		Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Apparent assimilation calculated for 50 sq. cms. and 1 hour.	Real assimilation* calculated for 50 sq. cms. and 1 hour.
		Time.	Temp.						
Experiment XXXI.—April 25, 1902. 11°4 C.									
Unit.	Area, 45.1 sq. centims. 1st weight, 1.65 grammes.	9.15 P.M. 1 A.M.	11°4 11°4	9.10-10.30 P.M. 10.30-12.30 A.M. 12.30-2.30 " 2.30-4.30 " 4.30-6.30 " 6.30-8.30 "	Preliminary -0806½ -0812 -0809½ -0811½ -0806½	Preliminary -0276½ -0275 -0275½ -0270 -0268½	— -0081 -0087 -0084 -0041½ -0088	— -0017 -0080½ -0019 -0083 -0021	— -0019 -0022½ -0021 -0025 -0023
Sixfold at 9.15 A.M.		9.15 A.M. 1 P.M.	11°4 11°4	9.15-10.25 A.M. 10.25-12.25 P.M. 12.25-2.25 "	Preliminary -0825 -0818	Preliminary -0237½ -0280	— -0087½ -0088	— -0048½ -0049	— -0050½ -0051
Fourfold at 2.30 P.M.	2nd weight, 1.70 grammes.	6 P.M. 8 "	11°4 11°4	2.30-3.15 P.M. 3.15-5.15 " 5.15-7.15 "	Preliminary -0816½ -0812	Preliminary -0236½ -0231	— -0081 -0081	— -0045 -0045	— -0046½ -0046½
Experiment XXXII.—April 26, 1902. 11°4 C.									
Unit.	Area, 36.6 sq. centims. 1st weight, 1.50 grammes.	9 P.M. Midnight	11°4 11°4	9.0-10.40 P.M. 10.40-12.40 A.M. 12.40-2.40 " 2.40-4.40 " 4.40-6.40 " 6.40-8.40 "	Preliminary -0808½ -0808 -0807 -0806 —	Preliminary -0281½ -0279½ -0277 -0275 —	— -0027 -0028½ -0080 -0081	— -0018½ -0019½ -0020½ -0021	— -0020½ -0021½ -0022½ -0023 —
Twofold at 8.40 A.M.	2nd weight, 1.50 grammes.	8.40 A.M. 11.0 " 5.40 P.M.	11°4 11°4 11°4	8.40-9.40 A.M. 9.40-11.40 " 11.40-1.40 P.M. 1.40-3.40 " 3.40-5.40 "	Preliminary -0804½ -0814 -0815 -0816	Preliminary -0254 -0261 -0266 -0262½	— -0050½ -0058 -0049 -0058½	— -0085 -0086 -0083½ -0086½	— -0087 -0088 -0085½ -0088½
Experiment XXXIII.—April 24 and 25, 1902. 11°4 C.									
Intensity = 1.37.	Area, 46.5 sq. centims. 1st weight, 1.65 grammes.	6.45 P.M. 10.0 " 1.0 A.M. 2.45 " 4.45 " 8.15 "	11°4 11°4 11°4 11°4 11°5	6.45-8.45 P.M. 8.45-10.45 " 10.45-12.45 A.M. 12.45-2.45 " 2.45-4.45 " 4.45-6.45 " 6.45-8.45 "	Preliminary -0809½ -0810 -0808½ — —	Preliminary -0250 -0250 -0258½ — —	— -0050½ -0051 -0050 — —	— -0027 -0027½ -0027 — —	— -0029 -0029½ -0029 — —
Fourfold at 10.15 A.M.	2nd weight, 1.72 grammes.	10.30 A.M. 12.0 noon 8.30 P.M.	11°3 11°3	8.45-11.25 A.M. 11.25-1.25 P.M. 1.25-3.25 "	Preliminary -0885 -0840½	Preliminary -0252½ -0257½	— -0082½ -0088	— -0045 -0045	— -0046½ -0046½

* Respiration calculated from Table I.

TABLE V.—*continued.*

Intensity of light.	Weight and area.	Temperature.		Time.	CO ₂ supplied. Control tubes.	CO ₂ un-assimilated. Tubes from chamber.	Apparent assimilation. Difference.	Apparent assimilation* calculated for 50 sq. cms. and 1 hour.	Real assimilation* calculated for 50 sq. cms. and 1 hour.
		Time.	Temp.						
Experiment XXXIV.—April 29, 1902. 25°·2 C.									
Unit.	Area, 41·5 sq. centims. 1st weight, 1·60 grammes.	10.15 A.M. 12.0 noon 1.15 P.M.	25°2 25°2 25°2	10.15–11.15 A.M. 11.15–12.15 P.M. 12.15– 1.15 "	Preliminary ·0159½ ·0152	Preliminary ·0146½ ·0139	— ·0018 ·0013	— ·0015½ ·0016½	— ·0021½ ·0021½
Fourfold at 1.15 P.M.		1.45 P.M. 3. 0 " 4. 0 "	25°2 25°2 25°2	1.15–2.0 P.M. 2. 0–3.0 " 3. 0–4.0 "	Preliminary ·0146½ ·0152	Preliminary ·0095½ ·0108	— ·0048 ·0044	— ·0053 ·0053	— ·0064 ·0059
Experiment XXXV.—April 30, 1902. 25°·2 C.									
Unit.	Area, 40·0 sq. centims. 1st weight, 1·75 grammes.			12.0–3.0 P.M.			No readings taken.		
Twofold at 3 P.M.		3.0 P.M. 5.0 "	25°·2 25° 2	3.0–4.0 P.M. 4.0–5.0 " 5.0–6.0 "	Preliminary ·0125 ·0123	Preliminary ·0093½ ·0098	— ·0026½ ·0024	— ·0039 ·0030½	— ·0040 ·0036½
Experiment XXXVI.—April 19 and 20, 1902. 24°·8 C.									
Fourfold.	Area, 39·2 sq. centims. 1st weight, 1·54 grammes.	1. 0 P.M. 2. 0 " 6.45 "	24°8 24°8 24°8	12.0– 2.0 P.M. 2.0– 4.0 " 4.0– 6.0 " 6.0– 8.0 " 8.0–10.0 "	Preliminary ·0902 ·0801½ ·0801 ·0801 ·0298	Preliminary ·0285½ ·0283½ ·0286½ ·0281½ ·0286½	— ·0066½ ·0063 ·0064½ ·0066½	— ·0042½ ·0043½ ·0041 ·0042½	— ·0048 ·0049½ ·0047½ ·0048
Unit at 10 P.M.		10.15 P.M.	24°8	10.0–12.0 night 12.0– 2.0 A.M. 2.0– 4.0 " 4.0– 6.0 " 6.0– 8.0 " 8.0–10.0 "	Preliminary ·0295 ·0290½ ·0805 ·0801 ·0293	Preliminary ·0278½ ·0282½ ·0290 ·0281½ ·0279	— ·0016½ ·0017 ·0015 ·0019½ ·0019	— ·0010½ ·0011 ·0009½ ·0012½ ·0012	— ·0017 ·0017½ ·0016 ·0020 ·0019½
	2nd weight, 1·60 grammes.	10.0 A.M.	25°0						

Respiration calculated from Table I

TABLE VI.—Summary of the Experiments in Table V., setting forth the Ratios of the Assimilation with the different Intensities of Light. [The Absolute Amounts of Assimilation given are the Means of the Readings in the Respective Experiments of Table V.]

Number of experiment.	Temp.	Apparent assimilation 50 sq. centims. 1 hour.				Real assimilation 50 sq. centims. 1 hour.				Ratios of real assimilations.			
		L.In. = 1.		L.In. = 2.		L.In. = 4.		L.In. = 6.		L.In. = 1.		L.In. = 2.	
		L.In. = 1.	L.In. = 2.	L.In. = 1.	L.In. = 2.	L.In. = 1.	L.In. = 2.	L.In. = 1.	L.In. = 2.	L.In. = 1.	L.In. = 2.	L.In. = 1.	L.In. = 2.
XXVIII.	0° 4 C.	·0017½	·0020	—	—	·0017½	·0020	—	—	1	1·14	—	—
XXIX.	8° 8 C.	·0020½	·0036½	—	—	·0022½	·0038½	—	—	1	1·71	—	—
XXX.	9° 2 C.	·0018½	—	·0035	—	·0020½	—	·0036½	—	1	—	1·78	—
XXXI.	11° 4 C.	·0020	—	·0045	·0049	·0022	—	·0046½	·0050½	1	—	2·16	2·34
XXXII.	11° 4 C.	·0020	·0035	—	—	·0022	·0037½	—	—	1	1·70	—	—
XXXIII.	11° 4 C.	—	—	·0045	—	—	—	·0046½	—	—	—	—	—
XXXIV.	25° 2 C.	·0015½	—	·0055½	—	·0021½	—	·0061½	·0063	1	—	2·88	—
XXXV.	25° 2 C.	—	·0032	—	—	—	·0038½	—	—	—	1·80	—	—
XXXVI.	24° 8 C.	·0013	—	·0042½	—	·0018½	—	·0048½	—	1	—	2·80	—

VEGETABLE ASSIMILATION AND RESPIRATION.

TABLE VII.

Assimilation experiments at medium and high temperatures with light of eightfold intensity (L.In. = 8). Two ordinary incandescent burners at low gas pressure. New mantles for each experiment.

Illumina- tion.	Weight and area.	Temperature.		Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimila- tion per 2 hours.	Respira- tion per 2 hours.	Real assimila- tion per hour per 50 sq. cms.
		Time.	Temp.						
Experiment XXXVII.—March 4, 1903. 11°·0 C.									
Light.	Area, 40·0 sq. centims. Weight, 1·50 grammes.	11° C. throughout.		10·0 - 11·30 A.M.	Preliminary	Preliminary	—	—	—
				11·30 - 1·30 P.M.	·0366	·0258	·0108	—	
				1·30 - 3·30 "	·0378	·0270	·0108	—	
				3·30 - 5·30 "	·0368	·0260	·0108	—	
				Mean values per hour		·0054	·0002*	·0072	
Intensity changed to L.In. = 4·6.				5·30 - 7·30 P.M.	Preliminary	Preliminary	—	—	—
				7·30 - 9·30 "	·0377	·0275	·0102	—	
				9·30 - 11·30 "	·0380	·0288	·0097	—	
				Mean values per hour		·0050	·0002*	·0066	

Experiment XXXVIII.—January 29 and 30, 1903. 25°·4 C.

Darkened.	Area, 41·0 sq. centims. 1st weight, 1·70 grammes.	10 A.M.	25·3	10·0 - 11·30 A.M. 11·30 - 1·30 P.M. 1·30 - 3·30 "	Preliminary ·0225 ·0220	Preliminary ·0233½ ·0226	— ·0008½ ·0006		
Light 4 P.M.		4 P.M.	25·3	4·0 - 6·0 P.M.	Preliminary ·0416	Preliminary ·0129	— ·0288	—	
		11·30 P.M. midnight.	25·4	6·0 - 9·0 " 9·0 - 12·0 night	·0427 ·0410	·0160 ·0168	·0267 ·0242	—	
	25·7		12·0 - 3·0 A.M. 3·0 - 6·0 " 6·0 - 9·0 "	·0419 ·0403	·0184 ·0199	·0285 ·0204	—		
	Darkened.		9 A.M.	25·4	9·0 - 11·0 A.M. 11·0 - 1·0 P.M. 1·0 - 3·0 " 3·0 - 5·0 "	Preliminary ·0230 ·0228 ·0222	Preliminary ·0272 ·0266 ·0258	— ·0042 ·0036 ·0036	
	2nd weight, 1·75 grammes.								
Mean values per hour							·0123½	·0011½	·0128

* Calculated from Table I.

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TABLE VII.—*continued.*

Illumination.	Weight and area.	Temperature.		Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimilation per 2 hours.	Respiration per 2 hours.	Real assimilation per hour per 50 sq. cms.
		Time.	Temp.						
Experiment XXXIX.—January 31, 1903. 25°·3 C.									
Darkened.	Area, 37·2 sq. centims. 1st weight, 1·50 grammes.	10 A.M.	25°·1	10-11.30 A.M. 11.30-1.30 P.M. 1.30-3.30 "	Preliminary ·0333 ·0340	Preliminary ·0352 ·0346		— ·0019 ·0006	
Light 4 P.M.		4 P.M. 5 P.M.	25°·1 25°·5	4-6 P.M. 6-8 " 8-10 " 10-12 night	Preliminary ·0351 ·0373 ·0372	Preliminary ·0166 ·0213 ·0215	— ·0175 ·0155 ·0157	— — — —	— — — —
Darkened midnight.	2nd weight, 1·55 grammes.	9 A.M.	25°·5	12-2 A.M. 2-4 " 4-6 "	Preliminary ·0362 ·0356	Preliminary ·0362 ·0392		— ·0030 ·0036	
					Mean values per hour		·0081	·0011½	·0125
Experiment XL.—February 4, 1903. 32°·1 C.									
Darkened.	Area 40·0 sq. centims. 1st weight, 1·52 grammes.	10.30 A.M. 12 noon	32°·1 32°·1	10.30-12 noon 12-2 P.M. 2-4 "	Preliminary ·0387 ·0396	Preliminary ·0372 ·0362		— ·0035 ·0026	
Light 4 P.M.		4 P.M. 6 P.M.	32°·1 32°·5	4-6 P.M. 6-8 " 8-10 " 10-12 night	Preliminary ·0353 ·0368 ·0356	Preliminary ·0173 ·0168 ·0169	— ·0180 ·0180 ·0167	— — — —	— — — —
Darkened midnight.	2nd weight, 1·52 grammes.	9 A.M.	32°·1	12-2 A.M. 2-4 " 4-6 "	Preliminary ·0354 ·0361	Preliminary ·0398 ·0398		— ·0042 ·0035	
					Mean values per hour		·0096	·0018½	·0129½
Experiment XLI.—February 5, 1903. 32°·2 C.									
Darkened.	Area 35·0 sq. centims. 1st weight, 1·40 grammes.	10 A.M. 1 P.M.	32°·1 32°·1	10-11.30 A.M. 11.30-1.30 P.M. 1.30-3.30 "	Preliminary ·0384 ·0386	Preliminary ·0368 ·0351		— ·0029 ·0015	
Light 3.30 P.M.		4 P.M. 5.30 P.M.	32°·2 32°·3	3.30-5.30 P.M. 5.30-7.30 " 7.30-9.30 " 9.30-11.30 "	Preliminary ·0321 ·0320 ·0315	Preliminary ·0169 ·0175 ·0167	— ·0152 ·0145 ·0148	— — — —	— — — —
Darkened 11.30 P.M.	2nd weight, 1·40 grammes.	9.30 A.M.	32°·3	11.30-1.30 A.M. 1.30-3.30 " 3.30-5.30 "	Preliminary ·0325 ·0315	Preliminary ·0370 ·0362		— ·0015 ·0047	
					Mean values per hour		·0074	·0017	·0130

TALLE VII.—continued.

Illumina- tion.	Weight and area.	Temperature.		Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimilation per 2 hours.	Respira- tion per 2 hours.	Real assimila- tion per hour per 50 sq. cms.
		Time.	Temp.						
Experiment XLIII.—February 2, 1903. 38° 3 C.									
Darkened.	Area, 38·7 sq. centims. 1st weight, 1·40 grammes.	10 A.M. 12 noon	38° 1 38° 1	10.0–11.30 A.M. 11.30– 1.30 P.M. 1.30– 3.30 "	Preliminary —0359 —0368	Preliminary —0402 —0400	— —0043 —0032	— — —	— — —
Light 3.30 P.M.		3.30 P.M. 5.30 "	38° 4 38° 4	3.30– 5.30 P.M. 5.30– 7.30 " 7.30– 9.30 " 9.30–11.30 "	Preliminary —0378 —0379 —0367	Preliminary —0242 —0281 —0240	— —0135 —0148 —0127	— — — —	— — — —
Darkened 11.30 P.M.	2nd weight, 1·40 grammes.	9 A.M.	38° 3	11.30– 1.30 A.M. 1.30– 3.30 " 3.30– 5.30 "	Preliminary —0356 —0347	Preliminary —0408 —0385	— —0052 —0038	— — —	— — —
					Mean values per hour		—0068½	—0020½	—0115

Experiment XLIII.—February 3, 1903. 38° 3 C.

Darkened.	Area, 89·7 sq. centims. 1st weight, 1·48 grammes.	10 A.M. 12 noon	85·3 85·3	10·0–11·30 A.M. 11·30–1·00 P.M. 1·30–3·30 „	Preliminary ·0825 ·0882	Preliminary ·0804 ·0862	— ·0089 ·0080	—	—
Light 3·30 P.M.		3·30 P.M. 5·30 „	88·3 88·5	3·30–5·30 P.M. 5·30–7·30 „ 7·30–9·30 „ 9·30–11·30 „	Preliminary ·0832 ·0887 ·0843	Preliminary ·0156 ·0172 ·0194	— ·0176 ·0155 ·0149	— — — —	— — — —
Darkened 11·30 P.M.	2nd weight, 1·45 grammes.	9·30 A.M.	88·3	11·30–1·30 A.M. 1·30–3·30 „ 3·30–5·30 „	Preliminary ·0352 ·0349	Preliminary ·0399 ·0404	— ·0047 ·0055	—	—
					Mean values per hour		·0081½	·0023	·0130

Experiment XLIV.—February 9, 1903. 40°·9 C.

Darkened.	Area, 41.4 sq. centims. 1st weight, 1.55 grammes.	40°-9 C. throughout experiment.	10.0 -11.30 A.M. 11.30 - 1.30 P.M. 1.30- 2.30 "	Preliminary '0895 —	Preliminary '0860 —	— '0025 —	
Light 3.30 P.M.			3.30-5.30 P.M. 5.30-7.30 " 7.30-9.30 " 9.30-11.30 "	Preliminary '0860 '0870 '0868	Preliminary '0237 '0262 '0233	— — '0123 '0103 —	— — — —
Darkened 11.30 P.M.	2nd weight, 1.55 grammes.		11.30-1.30 A.M. 1.30- 3.30 " 3.30- 5.30 "	Preliminary '0864 '0862	Preliminary '0893 '0857	— '0084 '0025	
				Mean values per hour		'0051½ '0014	'0078

TABLE VII.—*continued.*

Illumina- tion.	Weight and area.	Temperature.	Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimila- tion per 2 hours.	Respira- tion for 2 hours.	Real assimila- tion per hour per 50 sq. cms.
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Experiment XLV.—February 10, 1903. 40°·9 C.

Darkened.	Area, 40·0 sq. centims. Weight, 1·60 grammes.	40°·9 C. throughout experiment.	10.0–11.30 A.M. 11.30–1.30 P.M. 1.30–3.30 "	Preliminary ·0354 ·0361	Preliminary ·0379 ·0377	—	—	·0025 ·0016
Light 3.30 P.M.			3.30–5.30 P.M. 5.30–7.30 " 7.30–9.30 " 9.30–11.30 "	Preliminary ·0342 ·0349 ·0345	Preliminary ·0341 ·0240 ·0247	— ·0101 ·0109 —	— — — —	— — — —
Darkened 11.30 P.M.			11.30–1.30 A.M. 1.30–3.30 " 3.30–5.30 "	Preliminary ·0383 ·0383	Preliminary ·0379 ·0379	—	—	·0041 ·0046
				Mean values per hour		·0051	·0016	·0080

Experiment XLVI.—February 6, 1903. 42°·9 C.

Darkened.	Area, 29·0 sq. centims. 1st weight, 1·48 grammes.	42°·9 C. throughout experiment.	10.30–12.0 noon 12.0–2.0 P.M. 2.0–4.0 "	Preliminary ·0291 ·0294	Preliminary ·0327 ·0319	—	—	·0036 ·0025
Light 4 P.M.			4.0–6.0 P.M. 6.0–8.0 " 8.0–10.0 " 10.0–12.0 night	Preliminary ·0370 ·0337 ·0399	Preliminary ·0328 ·0355 ·0377	— ·0042 ·0032 ·0022	— — — —	— — — —
Darkened midnight.	2nd weight, 1·48 grammes.		12.0–2.0 A.M. 2.0–4.0 " 4.0–6.0 "	Preliminary ·0393 ·0382	Preliminary ·0423 ·0418	—	—	·0025 ·0036
				Mean values per hour		·0016	·0015	·0040

Experiment XLVII.—February 7, 1903. 42°·9 C.

Darkened.	Area, 39·2 sq. centims. 1st weight, 1·48 grammes.	42°·9 C. throughout experiment.	10.0–11.30 A.M. 11.30–1.30 P.M. 1.30–3.30 "	Preliminary ·0395 ·0391	Preliminary ·0425 ·0406	—	—	·0030 ·0015
Light 3.30 P.M.			3.30–5.30 P.M. 5.30–7.30 " 7.30–9.30 " 9.30–11.30 "	Preliminary ·0393 ·0412 ·0402	Preliminary ·0372 ·0375 ·0382	— ·0026 ·0037 ·0020	— — — —	— — — —
Darkened 11.30 P.M.	2nd weight, 1·48 grammes.		11.30–1.30 A.M. 1.30–3.30 " 3.30–5.30 "	Preliminary ·0396 ·0397	Preliminary ·0432 ·0416	—	—	·0036 ·0029
				Mean values per hour		·0014	·0014	·0036½

TABLE VIII.

Preliminary assimilation experiments with high intensity of light (L.In. = < 45)
Keith incandescent compound burner at high gas pressure (8 ins. water).

Weight and area.	Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimilation per hour.	Respiration per hour.	Real assimilation calculated for 1 hour and 50 sq. cms.
Weight, 1.50 grammes. Area 36.0 sq. centims.	10.15-11.45 A.M.	Preliminary	Preliminary	—		
	11.45-12.45 P.M.	•0980	•0315	•0065		
	12.45- 1.45 "	•0877	•0317	•0030		
	1.45- 2.45 "	•0382	•0313	•0069		
	2.45- 3.45 "	•0382	•0320	•0062		
		Mean values per hour. .		•0064	•0002*	•0090

Experiment XLVIII.—March 3, 1903. Bath temperature 11° C.

Experiment XLIX.—March 2, 1903. Bath temperature 29° C.						
Weight, 1.48 grammes. Area 37.0 sq. centims.	10.15-12.15 P.M.	Preliminary	Preliminary	—		
	12.15- 1.15 "	•0361	•0195	•0166	—	—
	1.15- 2.15 "	•0875	•0232	•0143	—	—
	2.15- 3.15 "	•0884	•0225	•0159	—	—
	3.15- 4.15 "	•0889	•0214	•0175	—	—
Darkened	4.15- 6.15 P.M.		Preliminary	—	—	—
	6.15- 8.15 "		•0025	—	•0012½	—
	8.15-10.15 "		•0022	—	•0011	—
	10.15-12.15 A.M.		•0024	—	•0012	—
		Mean values per hour. .		•0161	•0012	•0233

Experiment L.—February 27, 1903. Bath temperature 32° C.

Weight, 1.35 grammes. Area 37.0 sq. centims.	12.30- 1.30 P.M.	Preliminary	Preliminary	—	—	—
	1.30- 2.30 "	•0324	•0136	•0188	—	—
	2.30- 3.30 "	•0311	•0150	•0161	—	—
	3.30- 4.30 "	•0305	•0155	•0150	—	—
		Mean values per hour. .		•0166	•0010*	•0249

Experiment LI.—February 28, 1903. Bath temperature 38° C.

Weight, 1.55 grammes. Area 39.3 sq. centims.	11.20-12.45 P.M.	Preliminary	Preliminary	—	—	—
	12.45- 1.45 "	•0880	•0299	•0081	—	—
	1.45- 2.45 "	•0890	•0315	•0075	—	—
	2.45- 3.45 "	•0378	•0308	•0070	—	—
		Mean values per hour. .		•0075	•0019*	•0125

* Calculated from Table I.

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TABLE IX.

Further preliminary assimilation experiments with high intensity of light (L.In. = 74)
Keith incandescent compound burner at high gas pressure (8 ins. water).

Illumina- tion.	Weight and area.	Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimila- tion per hour.	Respira- tion per hour.	Real assimila- tion per hour per 50 sq. cms.
Experiment LII.—March 9, 1903. Bath temperature 32° C.							
L.In. = 74.	Area, 36.0 sq. centims. 1st weight, 1.40 grammes. 2nd weight, 1.50 grammes. [Leaf brown at centre.]	11.15-12.45 P.M. 12.45- 1.45 " 1.45- 2.45 "	Preliminary "0447 "0457	Preliminary "0867 "0402	— "0080 "0055	— — —	— — —
			Mean values per hour		"0068	"0010*	"0108
Experiment LIII.—March 10, 1903. Bath temperature 32°.							
L.In. = 81.	Weight, 1.10 grammes. Area, 34.8 sq. centims.	11.0 -12.30 P.M. 12.30- 1.30 " 1.30- 2.30 " 2.30- 3.30 "	Preliminary "0420 Spoilt "0428	Preliminary "0232 — "0320	— "0128 — "0108	— — — —	— — — —
L.In. = 74.		3.30- 4.30 P.M. 4.30- 5.30 " 5.30- 6.30 "	"0428 "0443 "0439	"0330 "0307 "0417	"0098 "0076 "0022	— — —	— — —
Darkened. CO ₂ free current.	[Leaf one quarter brown.]	6.30- 8.30 P.M. 8.30-10.30 " 10.30-12.30 A.M.		Preliminary "0020 "0021		— "0010 "0010½	
			Mean values per hour		"0086	"0010	"0129
Experiment LIV.—March 11, 1903. Bath temperature 11° C.							
L.In. = 74.	Weight, 1.55 grammes. Area, 36.7 sq. centims. [Leaf quite green.]	11.0 -12.30 P.M. 12.30- 1.30 " 1.30- 2.30 " 2.30- 3.30 " 3.30- 4.30 "	Preliminary "0433 "0495 "0485 "0490	Preliminary "0338 "0358 "0360 "0368	— "0150 "0137 "0125 "0122	— — — — —	— — — — —
			Mean values per hour		"0134	"0002*	"0176

* Calculated from Table I.

TABLE X.

An experiment with a Keith high pressure burner showing the unavoidable rise in the assimilation maximum that results, with each augmentation of the light intensity, from the decided increase of internal leaf temperature that the radiation produces, in spite of the bath temperature being kept constant throughout.

Illumination.	Weight and area.	Probable temperature.*	Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimilation per hour.	Real assimilation per hour per 50 sq. cms.†
Experiment LV. March 12-15, 1903.							
L.In. = 18.5	Weight, 1.40 grammes. Area 40.5 sq. centims.	18° C.	11.0-12.30 P.M. 12.30-1.30 " 1.30-2.30 " 2.30-3.30 "	Preliminary -0458 -0497 -0499	Preliminary -0398 -0426 -0422	-0085 -0071 -0077	-0100
L.In. = 9.25		16° C.	3.30-4.30 P.M. 4.30-5.30 " 5.30-6.30 " 6.30-7.30 "	Preliminary -0280 -0272 Spoilt	Preliminary -0290 -0225 Spoilt	-0060 -0047 -	-0068½
L.In. = 4.75		13° C.	7.30-8.55 P.M. 9.0-10.0 " 10.0-11.0 " 11.0-12.0 night	Preliminary -0498 -0505 -0492	Preliminary -0453 -0468 -0450	-0045 -0042 -0042	-0055½
Darkened all night. Relighted 9.40 A.M., March 13.							
L.In. = 4.75		18° C.	9.40-11.10 A.M. 11.10-12.10 P.M. 12.10-1.10 " 1.10-2.10 "	Preliminary -0400 -0420 -0432	Preliminary -0350 -0375 -0390	-0050 -0045 -0042	-0050
L.In. = 2.87		11° C.	2.10-3.10 P.M. 3.10-4.10 " 4.10-5.10 " 5.10-6.10 "	Preliminary -0441 -0468 -0445	Preliminary -0413 -0433 -0420	-0028 -0035 -0025	-0039
L.In. = 1.47		11° C.	6.10-7.10 P.M. 7.10-8.10 " 8.10-9.10 " 9.10-10.10 "	Preliminary -0447 -0447 -0443	Preliminary -0426 -0427 -0432	-0021 -0020 -0016	-0024
Darkened for 36 hours. Relighted 9.30 A.M., March 15.							
L.In. = 1.47		11° C.	9.30-11.30 A.M. 11.30-1.30 P.M. 1.30-3.30 "	Preliminary -0335 -0339	Preliminary -0293 -0284	-0042 -0055	-0030½
L.In. = .78		11° C.	3.30-4.30 P.M. 4.30-5.30 " 5.30-6.30 " 6.30-7.30 "	Preliminary -0323 -0335 -0321	Preliminary -0307 -0305 -0293	-0021 -0020 -0028	-0018½

* Based on determinations made later, see p. 76.

† Respiration for respective temperatures allowed from Table I.

TABLE XI.

Experiments showing maximal assimilation at temperatures between 15° C. and 40° C. Keith incandescent compound burner at high gas pressure (8 ins. water), and electrical determinations of internal leaf temperature.

Illumina- tion.	Weight and area.	Temperature.		Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimila- tion per hour.	Respira- tion per hour.	Real assimila- tion cal- culated for 50 sq. cms. and 1 hour.
		Time.	Temp.						
Experiment LVI.—April 6, 1903. 15° 0 C.									
L.In. = 13.	Area, 37.0 sq. centims. Weight, 1.50 grammes.	Internal tempera- ture of leaf, 15° C. Temperature of bath, 10° C.	10.30-12.10 P.M. 12.10- 1.10 " 1.10- 2.10 " 2.10- 3.10 " 3.10- 4.10 "	Preliminary -0356 -0352 -0353 -0360	Preliminary -0306 -0306 -0303 -0312½	— -0050 -0046 -0050 -0047½	— — — — —	— — — — —	— — — — —
Darkened 4.30 P.M. CO ₂ free current.		4.15 P.M. 15° 0 11 P.M. 15° 0	4.10- 6.0 " 6.0 -11.12 "		Preliminary -0017		— -0008½		
				Mean values per hour		-0048½	-0008½	-0070½	
Experiment LVII.—April 4, 1903. 23° 7 C.									
L.In. = 26.	Area, 42.0 sq. centims. Weight, 1.48 grammes.	Temperature of leaf, 23° 7 C. Temperature of bath, 11° C.	12.30- 2.0 P.M. 2.0 - 3.0 " 3.0 - 4.0 " 4.0 - 5.0 " 5.0 - 6.0 "	Preliminary -0382 -0418 -0374 -0325	Preliminary -0317 -0338 -0290 -0249	— -0055 -0080 -0084 -0076	— — — — —	— — — — —	— — — — —
Darkened 6 P.M. CO ₂ free current.		6.35 P.M. 23° 7 11.40 " 23° 7	6.0 - 7.30 P.M. 7.30- 9.30 " 9.30-11.30 "		Preliminary -0020½ -0015½		— -0010 -0008		
				Mean values per hour		-0076	-0009	-0101	
Experiment LVIII.—April 3, 1903. 30° 5 C.									
L.In. = 45.	Area, 45.0 sq. centims. Weight, 1.60 grammes.	Temperature of leaf, 30° 5 C. Temperature of bath, 15° 5 C.	10.0 -11.30 A.M. 11.30-12.30 P.M. 12.30- 1.30 " 1.30- 2.30 " 2.30- 3.30 "	Preliminary -0360 -0368 -0366 -0379	Preliminary -0226 -0270 -0260 -0280	— -0134 -0116 -0106 -0099	— — — — —	— — — — —	— -0157 — — —
Darkened 4 P.M. CO ₂ free current.		4.50 P.M. 30° 7 6.0 " 31° 3 midnight 31° 5	4.0 - 5.30 P.M. 5.30- 7.30 " 7.30- 9.30 " 9.30-11.30 "		Preliminary -0038 -0022 -0024		— -0011½ -0011 -0012		
				Mean values per hour		-0113½	-0011½	-0136	

VEGETABLE ASSIMILATION AND RESPIRATION.

TABLE XI.—*continued.*

Illumina- tion.	Weight and area.	Temperature.		Time.	CO ₂ supplied.	CO ₂ in current after leaving chamber.	Apparent assimila- tion per hour.	Respira- tion per hour.	Real assimila- tion cal- culated for 50 sq. cms and 1 hour.
		Time.	Temp.						
Experiment LIX.—April 7, 1903. 37°·5 C.									
L.In. = 45.	Weight, 1·48 grammes. Area, 38·0 sq. centims.	Internal tempera- ture of leaf, 37°·5 C. Temperature of bath, 28°·5 C.		10.30-12.0 noon 12.0 - 1.0 P.M. 1.0 - 2.0 " 2.0 - 3.0 " 3.0 - 4.0 "	Preliminary '0364 '0366 '0384½ '0409	Preliminary '0210 '0260 '0205 '0350	— '0154 0106 '0079½ '0059	— — — —	— '0237 — — —
Darkened 4.10 P.M. CO ₂ free current.		4.30 P.M. 5.30 " 11.30 "	37°·6 37°·4 37°·6	4.10- 5.30 P.M. 5.30- 7.30 " 7.30- 9.30 " 9.30-11.30 "	Preliminary '0040 '0042 '0036½		— '0020 '0021 '0018	— — —	— — —
					Mean values per hour		'0100	'0019	'0163
Experiment LX.—April 9, 1903. 40°·5 C.									
L.In. = 45.	Weight, 1·45 grammes. Area, 38·5 sq. centims.	Temperature of leaf, 40°·5 C. Temperature of bath, 32°·5 C.		2.50- 4.20 P.M. 4.20- 5.20 " 5.20- 6.20 " 6.20- 7.20 " 7.20- 8.20 "	Preliminary '0380 '0390 '0390 '0393	Preliminary '0281 '0315 '0330 '0373	— '0099 '0075 '0060 '0020	— — — —	— '0149 — — —
Darkened 8.30 P.M. CO ₂ free current.		8.30 P.M. 11.30 " 9.30 A.M.	40°·6 40°·5 40°·4	8.20- 9.50 P.M. 9.50-11.50 " 11.50- 1.50 A.M. 1.50- 3.50 "	Preliminary '0029 '0032 '0035		— '0014½ '0015½ '0017½	— — —	— — —
					Mean values per hour		'0063	'0016	'0102

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V. Cultural Experiments with "Biologic Forms" of the Erysiphaceæ.

By ERNEST S. SALMON, F.L.S.

Communicated by Professor H. MARSHALL WARD, F.R.S.

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1. Introductory.

DURING the past two years the existence of "biologic forms" in the *Erysiphaceæ* has been definitely proved. We know now that there exist in this group of parasitic fungi races of individuals which are morphologically identical, but which differ physiologically—or biologically—in possessing distinctive and sharply defined powers of infection. Thus we find a fungus A growing on host-plant *a*, and a fungus B growing on host-plant *b*. Now the fungus A belongs to the same species as fungus B, and is identical in every morphological character, yet the fungus A cannot infect the host-plant *b*, nor can the fungus B infect the host-plant *a*. The two fungi A and B thus prove to be "biologic forms" of the same morphological species.

This specialization of parasitism was first observed, (1) (2) (5), as regards the *Erysiphaceæ*, in the conidial (*Oidium*) stage of these fungi. At the beginning of the present year I published a paper "On the Infection-powers of the Ascospores in the *Erysiphaceæ*" (4), in which I gave the results of experiments which proved that the ascospores of "biologic forms" are similarly specialized in their powers of infection. Soon afterwards Professor E. MARCHAL published a summary of experiments in which the same results were obtained (3).

Since, then, restriction in power of infection to one species of host-plant, or to a group of closely allied species, has been proved to exist in these fungi in the sexual ascigerous stage as well as in the asexual conidial stage, we must admit the claim of these "biologic forms" to be considered as distinct entities.

2. The Behaviour of "Biologic Forms" under Certain Methods of Culture.

In the course of experiments carried on during the past summer, I have found that the restriction in power of infection characteristic of "biologic forms" breaks down if the vitality of the leaf on which the conidia are sown is interfered with in certain ways.

The first method of culture adopted was the following. The leaf to be inoculated was cut off from the plant, and a minute piece of the leaf-tissue was cut out with a razor. In this operation the epidermal cells on one surface, and all or most of the mesophyll tissue, were removed at the cut place, but the epidermal cells on the other surface (opposite the cut) were left uninjured. By this means a transparent window-like spot, 1-2 millims. across, was made in the leaf. The conidia were sown on the cuticular surface of the uninjured epidermal cells over the wound, and the leaf placed, with the cut surface downwards, on damp blotting-paper at the bottom of a closed Petri dish. As the epidermal cells on which the conidia were sown were rendered transparent by the removal of the underlying tissue, the development of the fungus could be followed in detail under the microscope.

Using this mode of culture, over fifty successful experiments were made in which the conidia of certain "biologic forms" were sown on "cut" leaves* of species which are normally immune to their attacks. The following case may be given to illustrate the point. If the conidia of *Erysiphe Graminis* occurring on wheat are sown on uninjured leaves of wheat and of barley, the result will be the infection of wheat but never of barley. That is to say, the form of *E. Graminis* on wheat is a "biologic form" confined under usual conditions to a few species of the genus *Triticum*. But if now we take the conidia of this "biologic form" on wheat and sow them on barley leaves which have been "cut" as described above, full infection results, and conidiophores with ripe conidia are produced in 6-8 days.

Other experiments described below seem to prove, further, that plants which are immune in nature can be artificially rendered susceptible. For instance, the two species of wheat, *Triticum monococcum* and *T. dicoccum* have been stated to be immune against mildew, both in the field and in laboratory infection-experiments (2). If, however, leaves of these two species are "cut" as described above, and inoculated with the conidia of the "biologic form" of *E. Graminis* on wheat, or even of a species of barley (*Hordeum sylvaticum*), infection readily occurs, and ripe conidiophores are produced in 4-6 days.

A summary of the experiments with "cut" leaves is given below (p. 114). The experiments described in this summary give conclusive evidence that under certain cultural conditions the distinctive characteristics of "biologic forms" break down, or in other words, that the range of infection of "biologic forms" becomes increased when the vitality of the leaf on which the conidia are sown is affected by injury.

It may be noted here that the conidia of the fungus produced on the "cut" leaf possess full germinating powers. For instance, conidia of the first generation produced on "cut" leaves of *Hordeum sylvaticum* by inoculation with conidia occurring on *H. vulgare*† readily infected "cut" leaves of *H. sylvaticum*; the conidia

* "Cut" leaves = leaves cut as described above. The word "cut" is used to designate the whole area from which mesophyll has been removed.

† All attempts to infect uninjured leaves of *H. sylvaticum* with conidia from *H. vulgare* failed.

thus obtained again readily infected "cut" leaves of *H. sylvaticum*, and so on to the third generation. Again, the conidia of the first generation produced on "cut" leaves of *H. sylvaticum* by inoculation with conidia occurring on *Triticum vulgare* infected, in eleven cases out of twenty-two, "cut" leaves of *T. vulgare*.

In all the experiments hitherto mentioned, leaves removed from the plant, and injured by "cuts," were used, and the question naturally presented itself: Was the susceptibility shown under the above conditions caused directly by the injury due to the "cut," or was it, in part at any rate, caused by the cutting off of the leaf from the plant?

Two series of experiments were carried out with the object of answering this question,

In the first, leaves of barley attached to plants were "cut" and inoculated with conidia from wheat. In one experiment a leaf of a plant of barley, 7 days old, was "cut" in two places (one each side of the midrib) and over one "cut" conidia from *Triticum vulgare* were sown, the other "cut" serving as a control. This "cut" leaf, still attached to the plant, growing in a pot, was then inserted through a hole into the bottom of a closed Petri dish and kept pressed down on wet blotting-paper. On the fifth day mycelial hyphæ and large lobed haustoria were evident at the place of inoculation over the "cut." By the tenth day, five conidiophores with chains of ripe conidia, and numerous younger conidiophores, had been produced; while the control "cut" showed no trace of any fungus. The leaf was now beginning to turn yellow towards the apex. On the seventeenth day the leaf was nearly dead throughout its length; three conidiophores were still visible at the inoculated spot, while the control "cut" was still free. In another experiment two "cuts" were made on one barley leaf attached to a plant in a pot, and conidia from *Triticum vulgare* were sown over both "cuts," the other plants in the pot serving as controls. The "cut" leaf was inserted into a closed Petri dish in the same manner as before. On the fourth day mycelial hyphæ had developed over both "cuts"; on the tenth day three conidiophores were formed; on the sixteenth ten conidiophores were visible over one "cut," and six over the other; the leaf-tissue surrounding the "cuts" was now turning yellow. By the twelfth day the leaf in its upper two-thirds was nearly colourless and almost dead, a few conidiophores were still visible; no signs of any fungus appeared elsewhere on the "cut" leaf, nor on the control leaves in the pot. In a third experiment a "cut" was made on a growing barley leaf attached to a young plant in a pot, and a drop of water placed on the "cut." On the dry epidermis over the "cut" conidia of the "biologic form" on wheat were sown. Conidia of the same "biologic form" were sown also on the epidermis of another uninjured leaf of the same plant. The pot was then placed under a bell-jar. On the sixth day the "cut" leaf, which still retained the drop of water hanging in the concavity of the "cut," was infected virulently with vigorous mycelial hyphæ and numerous large lobed haustoria. On the ninth day the "cut" leaf bore a few conidiophores. No infection resulted on the uninjured leaf.

In the second set of experiments leaves of barley and wheat were removed from growing plants, and some of each species placed, uninjured, in Petri dishes under the same conditions as in previous experiments. Conidia of the "biologic forms" on wheat and barley were sown at marked places on these leaves. No infection resulted when the conidia from wheat were sown on barley, nor in the reverse experiment, while infection never failed to occur on the wheat and barley leaves when conidia of the respective "biologic form" were sown.

It seems clear, therefore, that the effect caused by the "cut" on the vitality of the leaf is the factor which brings about increased susceptibility of the leaf to the attacks of the fungus.

It still remained to be seen whether the conidia produced on a "cut" leaf by inoculation with conidia of a "biologic form" which is unable to infect uninjured leaves of that species, possessed the power of infecting uninjured leaves of the plant in question; or whether the restricted infection-powers characteristic of the "biologic form" are retained, under these conditions, in the conidia of the first generation. The following experiment was made to determine this point. Conidia obtained on "cut" leaves of *Triticum vulgare* by inoculation with conidia of the *Oidium* occurring on *Hordeum sylvaticum* were sown on the uninjured damp upper surface of two leaves of *Triticum vulgare* attached to growing plants in a pot. On one leaf fairly numerous conidia were deposited; on the other only a very few. The inoculated plants were kept continuously covered over with a bell-jar for 6 days. The two leaves were then examined, and it was found that the leaf which had been sown with numerous conidia now bore at the place of inoculation a well-grown patch of mycelium, with a group of densely clustered conidiophores—about fifty in number—with chains of ripe conidia. The control leaves (six) were all free. By the ninth day a large and very vigorous *Oidium*-patch, powdery with masses of ripe conidia, was produced on this inoculated leaf. No infection resulted on the other inoculated leaf.

Further, conidia obtained on "cut" leaves of *Hordeum sylvaticum* by inoculation with conidia of the "biologic form" on *H. vulgare* were sown on the under surface, between inked lines, of two leaves of *H. sylvaticum* removed from the growing plant, but otherwise uninjured. These two inoculated leaves were placed by the side of two control leaves on damp blotting-paper in a Petri dish. On the ninth day the two inoculated leaves were covered continuously over the space between the inked lines (= 0.5 centim. wide) with vigorous mycelium bearing densely clustered conidiophores and powdery masses of ripe conidia. The two control leaves remained free.

A variation was now made in the method of culture. In three experiments conidia of *E. Graminis* were sown on leaves which had been "cut" as described above, but the conidia were now sown on the internal tissues of the leaf exposed by the cut.

In the first experiment, thirteen "cut" leaves of barley were inoculated with

conidia of the "biologic form" on barley. In some cases the leaves were "cut," and the conidia were at once sown on the cut surface, which was moist with sap exuding from the cut cells; in others, the cut surface was dried by exposure to air in a Petri dish for 8 hours. By the fifth day all thirteen leaves were virulently infected, the cut surface being covered by interwoven hyphæ bearing numerous little groups of conidiophores.

In the two other experiments conidia of the "biologic form" on wheat were sown on ten "cut" leaves of barley, on the cut surface, and placed by the side of six control leaves of barley, "cut," but not inoculated, on damp blotting-paper in a closed Petri dish. On the ninth day eight of the inoculated leaves were infected at the "cut"; all bore patches of mycelial hyphæ on the surface of the cut cells, and on five leaves the fungus had produced a little group of conidiophores. In some cases the group of conidiophores sprang from the internal surface (exposed by the cut) of the epidermal cells; in other cases from the exposed mesophyll tissue. The controls were everywhere quite free.

At Professor MARSHALL WARD's suggestion an experiment was now made to see if the same result, *i.e.*, the infection of the host-plant of one "biologic form" by the conidia of another "biologic form," could be obtained by a method which avoided lesion of the leaf. For this purpose eight barley leaves, which had been removed from the plant, were touched on the upper surface for a few seconds with a red-hot knife. These were then sown with the conidia of the "biologic form" of *E. Graminis* on wheat; on three leaves the conidia were placed on the burnt spot on the upper surface of the leaf; on five leaves on the lower surface opposite to the burnt spot. The leaves soon showed the effect of the application of the red-hot knife, a small area of leaf-tissue becoming discoloured, limp, and apparently dead on both surfaces. On the eighth day two of the three leaves bore at the burnt place little flecks of mycelium with a few conidiophores. The flecks of mycelium were at the very edge of the patch of killed cells on what appeared superficially to be uninjured cells. Of the five leaves each one was infected, and bore small flecks of mycelium with a few conidiophores. The mycelium occurred, as a rule, on living tissue at the very edge of the group of dead cells killed by the hot knife, but in one or two cases mycelial hyphæ could be seen on the now dead cells, and extending thence to the surrounding living tissue. No signs of any fungus appeared elsewhere on these seven leaves.

3. General Considerations.

One of the first questions that presents itself in connection with the present subject is: Do we meet, *in nature*, with injuries to leaves, such as those caused in the above experiments? A case which has come under my observation leads me to conclude that such injuries do occur, and shows also of what nature the injury may be. This case is as follows. In the course of infection-experiments with the "biologic forms"

of *E. Graminis* on species of *Bromus*, I have found that the "biologic forms" occurring on *B. commutatus* and *B. velutinus* are not able to infect *B. racemosus*. In these experiments, fifty leaves—from fifty different plants—of *B. racemosus* were inoculated, twenty-five with conidia from *B. commutatus*, and twenty-five with conidia from *B. velutinus*; fifty control leaves of *B. commutatus* and *B. velutinus* being inoculated at the same time. In every case, infection resulted on *B. commutatus* and *B. velutinus*, and failed on *B. racemosus*. There is no doubt, therefore, that we have on *B. commutatus* and *B. velutinus* a "biologic form" which is incapable under ordinary conditions of growing on *B. racemosus*. Now, these plants of *B. racemosus* were kept in the laboratory for 6 weeks or so during the experiment, and, at the end of this time, became infested with "green fly" (*Aphis*). These insects, in sucking the juices of the plant, injure the leaf in much the same manner as was done artificially by "cutting" the leaf, that is, by puncturing the leaf they destroy a few of the leaf-cells at little isolated spots. At these injured places on the leaf flecks of mycelium appeared, which eventually produced a few conidiophores. It seems to me that we have here a case of a plant which is immune against a fungus so long as the leaves remain uninjured, but which becomes susceptible when its leaves are injured by an insect.

Hitherto, the result of all experiments with "biologic forms" has been the accumulation of evidence tending to emphasise the fixity and sharply defined nature of "biologic forms." The evidence obtained was compelling us to believe that the numerous "biologic forms," of which we found each morphological species to be composed, were rigidly separated one from the other by immovable limits which restricted each "biologic form" either to a single host-species, or to certain host-species of one genus. At the most, a "biologic form" might, by means of a "bridging species" (see 5, p. 285), pass from the host-species of one section of a genus to those of another. The result of the present experiments, however, shows us some conditions under which "biologic forms" break down—some conditions under which the conidia of one "biologic form" can infect host-plants (of another "biologic form"), which are, as a rule, immune against its attacks. We find that when the vitality of the leaf-cells of a host-species, susceptible, as a rule, to the attacks of only one "biologic form," is affected by an injury—such as a cut or burn—the leaf-cells in the neighbourhood of the injury are rendered susceptible to the attacks of other "biologic forms." It is evident, and we have clear experimental proof of the fact, that such injured leaves will serve as "bridges," enabling a "biologic form," restricted in its parasitism, as a rule, to one genus, to pass over to the host-species of another genus.

We can see, now, more of the factors at work in the evolution of "biologic forms." We find, on the one hand, factors, which we may call "specialising factors," tending to make specialisation of parasitism more and more complete, with the result that there originate from each morphological species a number of "biologic forms," each one of which is incapable of infecting healthy leaves of any but its own host-species

or group of host-species. This breaking-up of a morphological species into "biologic forms" rigidly fixed in the sexual (ascosporic) stage as well as in the asexual (conidial) stage, would, we may believe, lead ultimately to the evolution of new *morphological* varieties or species. I have already (5, p. 264) pointed out that some "biologic forms" show in the conidial stage distinctive characteristics in the colour of the conidia, and, in some cases, a further difference is found in the size of the conidia.

But we find antagonistic factors at work, which we may term "generalising factors." When the vitality of the leaf-cells of a host-plant is affected by injury in certain ways, other "biologic forms" than the one specially adapted to it become capable of infecting it, and, as experiments have shown, conidia produced on these injured places are at once capable of infecting uninjured leaves of the same species. Thus the separate streams of evolving "biologic forms" are liable to flow into each other.

As to the actual manner in which the injury to a leaf causes it to become susceptible to a "biologic form" otherwise unable to infect it, the following explanation may be given tentatively. The phenomena of adaptive parasitism shown by the *Erysiphaceæ* so closely resemble those found in the *Uredineæ* that the hypothesis put forward by MARSHALL WARD (6) (7) as to the factors governing infection and immunity may be advanced for the *Erysiphaceæ* also. This hypothesis assumes the existence of enzymes or toxins, or both, in the cells of the fungus, and of anti-toxins, or similar substances, in the cells of the host-plant.

Now, in connection with the above experiments, we may suppose that the leaf-cells of wheat, barley, &c., contain substances peculiar to each species, which when the cells are vigorous and the leaf is uninjured, are able to prevent the successful attack of any mildew except the one "biologic form" which has become specialised to overcome the resistance. When the vitality of the cells, however, becomes affected by injury, either these substances are destroyed, or become weakened, or the production of them by the protoplasm is interfered with, in the leaf-cells in the neighbourhood of the injury, with the result that the conidia of other "biologic forms" are now able to infect them, since they no longer meet with any special substance able to injure them.

I would point out here that the present experiments furnish conclusive and direct proof that, as regards the *Erysiphaceæ*, the immunity of a certain host-species against the attacks of other "biologic forms" than its own in no way depends on any anatomical or structural peculiarities—such as hairs, ribs, thickness of the cell-wall or cuticle, or the chemical nature of the cell-wall, &c.—since in the experiments where a "cut" leaf became susceptible to a "biologic form" otherwise incapable of infecting it, the conidia were sown on the uninjured epidermis of the leaf.

It seems to me possible also that we may find in the facts shown by the above experiments the explanation of a phenomenon which so frequently confronts plant-

pathologists, viz., the sudden appearance of a parasitic fungus disease on plants which had hitherto proved immune. As the above experiments prove, it needs only an injury to be made to the leaves of certain plants in order to render them susceptible to disease, although otherwise they remain immune; and since, as experiments further proved, when the fungus has gained a footing on the injured parts, it can at once spread to uninjured parts, we can see how plants, the leaves of which have been injured by animals, hail, storms of winds, &c., may become the starting-point of a new disease.

It is worthy of note that the cases in which conidia of one "biologic form" were sown on the cut surface of the leaf of a host-species of another "biologic form," and proved under these conditions able to infect a plant otherwise immune to it, present a close and interesting parallel to the biological facts obtaining in the class of parasitic fungi known as "wound parasites"—*Polyporus*, *Botrytis*, *Nectria*, *Cucurbitaria*, *Peziza willkommii*, &c.—which are able to infect their hosts only through a wound.

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SUMMARY OF EXPERIMENTS.

1. *Experiments in which Conidia of one "Biologic Form" of E. Graminis were Sown on "Cut" Leaves of a Host-plant of another "Biologic Form."*

In all the following experiments conidia were sown on "cut" leaves belonging to a species whose uninjured leaves had been proved to be immune to these conidia.

Conidia from Triticum vulgare Sown on "Cut" Leaves of Hordeum vulgare.
(Experiments Nos. a, b, c, e-j.)

In these nine experiments fifty-eight leaves of *H. vulgare* were sown with conidia from *T. vulgare*, the leaves being "cut," and the method of culture adopted as described above. Infection resulted on forty-six of the leaves, the conidia producing patches of mycelium with little groups of conidiophores and ripe conidia on thirty-seven leaves, and on the remaining nine leaves patches of mycelium only.* Penetration of haustoria into the epidermal cells took place after 18 hours, and was at once followed by the vigorous growth of the first haustorium (which soon became lobed by the development of finger-like processes), and the production of hyphal branches from the appressorium of the germinating conidium. By about the

* The latter fact I believe to be due to these leaves dying before the fungus had time to produce conidiophores, and not to the occasional inability of the fungus to produce conidia under the conditions of culture used.

fourth day the bulbous bases of the first conidiophores were visible, and by 6-8 days little groups of conidiophores with chains of ripe conidia were formed. These persisted up to the tenth or eleventh day, sometimes becoming covered with little powdery heaps of accumulated ripe conidia. None of the thirty-six control leaves used in these experiments showed any signs of a fungus, and no fungus appeared on the "cut" leaves except at the place of inoculation. The leaves used in the experiments were taken from young seedling plants, and were usually at once "cut" and inoculated; in a few cases, however, the leaves were kept with their stalks in water 18 hours after being removed from the plant (in the afternoon). The leaves, which had become somewhat flaccid, were then "cut" and inoculated, and nine of fourteen leaves became infected—the fungus on seven of the leaves producing conidiophores. As a rule, the leaf-tissue in the neighbourhood of the "cut" began to change colour in 5-8 days, and the leaves in 11-15 days became yellowish or whitish throughout, and dead, or nearly dead. In some cases several "cuts," or even holes, were made in the neighbourhood of the inoculated place, and leaves so treated were as strongly infected—if not more so—as when only one "cut" was made. In some cases conidia were sown at marked places on uninjured leaves—removed from the plant, but not "cut"—and placed side by side with inoculated "cut" leaves. No infection resulted on the uninjured leaves. An uninjured wheat leaf, however, inoculated and treated in the same way, was virulently infected, and after eight days bore a powdery *Oidium*-patch. In one experiment, six "cut" leaves and six uncut leaves of barley were floated on water in a large Petri dish, and also two uncut wheat leaves. All the leaves were sown with conidia from wheat. On the seventh day five of the "cut" barley leaves showed vigorous patches of mycelium bearing little groups of conidiophores; no signs of infection occurred on the uncut barley leaves, while the wheat leaves were covered continuously at the marked place—1 centim. wide—with powdery *Oidium*-patches. In another experiment six pieces of barley leaves, $\frac{3}{4}$ -1 inch long, were "cut" and inoculated. By the sixth day the pieces of leaves had all turned yellow, and were nearly dead; three leaves, however, bore vigorous mycelial patches. On the eighth day one leaf bore a few conidiophores.

Conidia from T. vulgare Sown on "Cut" Leaves of Hordeum sylvaticum.
(Experiments Nos. s, z.)

Nine leaves were sown, and on four full infection took place, resulting in the production, in 5-6 days, of groups of conidiophores, with chains of ripe conidia. The fungus continued to grow vigorously, and gave rise, in 11-14 days, to luxuriant powdery *Oidium*-patches, which persisted for over 3 weeks. In one case, where three leaves were inoculated, a group of conidiophores appeared on one leaf exactly over the middle of the "cut," on the second leaf over the edge of the "cut," and on the third leaf at a spot about 1 millim. from it. (The leaves of this species of grass

possess the power of living for a long time after being removed from the plant. Leaves cut off and placed in a Petri dish on damp blotting-paper remain fresh and green for over 5 weeks.)

Conidia from T. vulgare Sown on "Cut" Leaves of Avena sativa.

(Experiment No. 11.)

Six leaves were inoculated. On the fourth day all the leaves had turned reddish-green, and were nearly dead. On two leaves lobed haustoria were formed, and small patches of mycelial hyphæ. The six control leaves were all free. No further growth of the fungus was observed, and on the eighth day the leaves were dead.

Conidia from Hordeum vulgare Sown on "Cut" Leaves of Triticum vulgare.

(Experiments Nos. d, k, l, v1.)

In the first experiment five "cut" leaves and five uninjured leaves of *T. vulgare* and one uninjured leaf of *Hordeum vulgare* were inoculated, and placed side by side in a large Petri dish. By the second day large lobed haustoria were formed on three of the five "cut" wheat leaves. On the seventh day four of the "cut" wheat leaves were infected with vigorous mycelial patches, one of which bore a few conidiophores; the uncut wheat leaves showed no signs of infection; while the uncut barley leaf was covered at the marked place with dense almost continuous *Oidium*-patches. In another case twelve leaves of *T. vulgare* were removed from seedling plants, and placed with the ends in water for 3 days. They were then all "cut," and six of them inoculated and placed side by side in a large Petri dish. On the second day penetration of haustoria had occurred in all the inoculated "cut" leaves. By the fourth day all the six inoculated leaves bore small mycelial patches over the "cuts"; no signs of any fungus were visible elsewhere on these leaves, nor on the six control leaves. By the eighth day all the leaves were nearly dead; mycelial patches were distinct on all the inoculated leaves, but no conidiophores were observed.

In the other experiments five uncut barley leaves and sixteen uncut wheat leaves were inoculated at marked places. By the sixth day all the barley leaves were virulently infected with *Oidium*-patches; no signs of infection appeared on the wheat leaves.

Conidia from H. vulgare Sown on "Cut" Leaves of H. murinum.

(Experiment No. n.)

Seven "cut" leaves were inoculated. After 18 hours penetration of haustoria had occurred on three leaves. At the end of 7 days all the leaves had turned yellow and were nearly dead; patches of interwoven mycelial hyphæ occurred on four leaves, but no conidiophores were observed.

Conidia from H. vulgare Sown on "Cut" Leaves of Avena sativa.

(Experiment No. k1.)

Six "cut" leaves were inoculated. By the fourth day all the leaves had turned

reddish-green and were nearly dead; on one leaf a small but vigorous patch of mycelial hyphæ occurred. Leaves dead on the eighth day.

Conidia from H. vulgare Sown on "Cut" Leaves of H. sylvaticum.

(Experiments Nos. 67*, 82*.)

In a previous infection-experiment (No. 67*b*) conidia of the "biologic form" on *H. vulgare* were sown on three uninjured leaves of growing plants (in a pot) of *H. sylvaticum*. No infection resulted. Twelve leaves from this pot of *H. sylvaticum* were then removed from the plants, and of these six were "cut" and sown with conidia from *H. vulgare*; the remaining six were not "cut" and were sown at marked places on the lower and upper epidermis with conidia from the same source—one of the originally inoculated leaves of Experiment 67*b* being used in each lot.

The twelve leaves were placed side by side in a large Petri dish. On the third day four of the six "cut" leaves were infected, numerous haustoria and hyphal branches proceeding from the appressorium being present; one of these infected leaves was a leaf previously used in Experiment 67*b*. On the seventh day all the six "cut" leaves showed vigorous mycelial patches. On the seventeenth day four of the "cut" leaves (including the leaf from Experiment 67*b*) showed groups of conidiophores.

No infection took place of the six uninjured cut-off leaves. In the second experiment three "cut" leaves of *H. sylvaticum* were inoculated. After 16 hours several haustoria had penetrated into the epidermal cells. On the fourth day the three leaves showed small patches of mycelium. On the sixth day small groups of conidiophores appeared on two of the leaves; by the eighth day little *Oidium*-patches powdery with conidia were visible over the "cuts" on all three leaves.

Conidia from H. sylvaticum Sown on "Cut" Leaves of Triticum vulgare.

(Experiments Nos. 81, 81.)

Seven leaves were taken direct from young seedling plants of *T. vulgare*, and "cut" and inoculated; three control leaves were placed at their side. On the fourth day virulent infection, as shown by the formation of very numerous large lobed haustoria and hyphal branches had taken place on five of the leaves over the "cuts." On the sixth day the mycelial patches on these five leaves bore little groups of densely clustered conidiophores, and on the tenth day *Oidium*-patches quite powdery with little masses of conidia. (These conidia were sown on an uninjured leaf of *T. vulgare*, and caused full infection.) No trace of any fungus appeared elsewhere on the "cut" leaves, or on the control leaves.

Conidia from Bromus commutatus Sown on "Cut" Leaves of Hordeum sylvaticum.

(Experiment No. 8.)

Seven leaves were inoculated. By the fifth day small patches of mycelial hyphæ were formed on two leaves. By the fifteenth day fairly vigorous but barren mycelial patches were visible on two leaves over the "cuts."

Conidia from B. commutatus Sown on "Cut" Leaves of B. racemosus.
(Experiment No. *a*1.)

Nine leaves were inoculated. On the seventh day the leaf-tissue was nearly dead in the neighbourhood of the "cut" places; on one leaf a distinct mycelial patch was evident over the "cut." By the tenth day all the leaves were dead.

Conidia from B. commutatus Sown on "Cut" Leaves of B. asper.
(Experiment No. *m*1.)

Twelve leaves were inoculated. On the fifth day the beginning of a mycelial patch was visible on one leaf. On the ninth day this leaf showed a vigorous patch of mycelium bearing a few conidiophores. On the eleventh day a few conidiophores were still visible on the one leaf, but all the leaves were nearly dead in the neighbourhood of the "cuts."

Conidia from B. secalinus Sown on "Cut" Leaves of Hordeum vulgare.
(Experiment No. *y*.)

Six leaves were inoculated. On the fourth day a small mycelial patch was visible on one leaf over the "cut." By the sixth day the patch of mycelium had increased, but all the leaves were beginning to die rapidly. On the ninth day all the leaves were dead.

Conidia from B. secalinus Sown on "Cut" Leaves of B. asper.
(Experiment No. *i*1.)

Five leaves were inoculated. On the third day one leaf bore a patch of mycelium; on the sixth day four young conidiophores appeared on this leaf, over the "cut"; the leaf-tissue in the neighbourhood of the "cuts" was dead on all other leaves. On the twelfth day the one infected leaf was also dead.

Conidia from B. velutinus Sown on "Cut" Leaves of Hordeum vulgare.
(Experiment No. *j*1.)

Twelve "cut" leaves were placed in a large Petri dish, and six inoculated. On the third day three leaves were infected, and showed numerous lobed haustoria and the beginnings of mycelial patches. On the fifth day these three leaves showed vigorous mycelial patches over the "cuts"; no fungus was visible elsewhere on these leaves, nor on the six controls. On the thirteenth day two of the infected leaves bore respectively two and three young conidiophores. No further growth was made by the fungus, in consequence, apparently, of the leaves now rapidly dying. By the eighteenth day all the leaves were dead.

Conidia from Avena strigosa Sown on "Cut" Leaves of Hordeum vulgare.

(Experiment No. u1.)

Seven leaves were inoculated. On the second day haustoria were visible on three leaves. On the fourth day a vigorous mycelial patch was formed on one leaf. By the sixth day three leaves bore vigorous patches of mycelium; no conidiophores were observed. On the ninth day the mycelial patches were still evident, but all the leaves were nearly dead.

Conidia from A. strigosa Sown on "Cut" Leaves of Triticum vulgare.

(Experiment No. u2.)

Seven leaves were inoculated. On the second day lobed haustoria formed on three leaves. On the fourth day vigorous mycelial patches formed on two leaves. By the sixth day these patches were overrun by other fungi, stopping further growth of the *Oidium*. On the ninth day the mycelial patches were all killed by the growth of other fungi.

2. *Experiments in which Conidia of E. Graminis were Sown on "Cut" Leaves of "Immune" Species of Plants.*

Conidia from Triticum vulgare Sown on "Cut" Leaves of T. dicoccum.

(Experiments Nos. m, u, w.)

In the first experiment six of the seven inoculated leaves became fully infected, and from the first the fungus behaved as though sown on its own host-species. On the second day virulent infection was shown on six leaves by the abundant formation of large lobed haustoria and the production of numerous hyphal branches. On the third day the bulbous bases of young conidiophores appeared on the mycelial patches, and on the fourth day groups of densely clustered ripe conidiophores. In the second experiment five of the eighteen inoculated leaves were infected and produced conidiophores. In the third, four of nine leaves were infected. The leaves of *T. dicoccum* die in 11-12 days, usually smelling foetidly of putrefaction. Frequently the infected spots remained of a vivid green colour when the rest of the leaf had turned pale yellow or brown.

Conidia from T. vulgare Sown on "Cut" Leaves of T. monococcum.

(Experiment No. p.)

Seven leaves were inoculated. On the second day lobed haustoria were formed on five leaves, and by the sixth day conidiophores, on vigorous mycelial patches, were produced on these leaves. By the fourth day the leaves began to die and turn brown round the "cuts," and by the eleventh all the leaves smelt of putrefaction and were nearly dead.

3. *Experiments in which Conidia produced on "Cut" Leaves were Sown on other "Cut" Leaves.*

Conidia Produced on "Cut" Leaves of Hordeum vulgare by Inoculation with Conidia from Triticum vulgare Sown on "Cut" Leaves of H. vulgare.
(Experiment No. o1.)

Six "cut" leaves were placed side by side, and three were inoculated (only a very few conidia were available for this experiment). On the sixth day a vigorous mycelial patch was formed over the "cut" on one leaf; the control leaves were all free. On the tenth day this patch bore two conidiophores with chains of ripe conidia, and several young conidiophores. On the sixteenth day a little group of conidiophores with an almost powdery mass of ripe conidia was visible on the one leaf; all the leaves were now rapidly dying.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from Triticum vulgare Sown on "Cut" Leaves of T. vulgare.
(Experiments Nos. b1, e1, g1, h1, r, r1.)

In these experiments twenty-two leaves were inoculated, and infection resulted on eleven leaves. In two cases quite powdery *Oidium*-patches appeared in 5-6 days on some of the inoculated leaves, and—all the experiments being safeguarded by controls—it is quite clear that under the conditions created the form of *R. Graminis* on *Hordeum sylvaticum* can fully and virulently infect *Triticum vulgare*. To illustrate the course of growth a summary of one experiment may be given here. Conidia of the fungus growing on "cut" leaves of *H. sylvaticum* (which had been inoculated on August 18 with conidia from *T. vulgare*, and which bore at the date of the present experiment—August 29—small powdery *Oidium*-patches) were sown on four "cut" leaves of *T. vulgare*, and four "cut" control leaves placed by their side. On the third day three of the inoculated leaves showed signs of virulent infection, the conidia having produced numerous lobed haustoria and hyphal branches. In fact, the virulence of infection shown here was as great as any observed in the Petri dish cultures. On the fifth day one leaf bore over the "cut" a large and vigorous *Oidium*-patch, quite powdery with little masses of ripe conidia; the two other leaves, also, bore each a smaller group of ripe conidiophores. All the controls remained perfectly free. On the eighth day two of the three infected leaves bore powdery *Oidium*-patches; the four controls showed no signs of a fungus. On the eleventh day all the leaves were dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from Triticum vulgare Sown on "Cut" Leaves of T. dicoccum.
(Experiment No. n1.)

Six "cut" leaves were placed side by side, and three inoculated. On the fifth day

two of the inoculated leaves bore over the "cuts" little groups of 3-7 conidiophores with chains of ripe conidia; the third inoculated leaf bore a vigorous patch of mycelial hyphæ. All the controls were perfectly free. On the eighth day all three leaves bore vigorous little patches of *Oidium* almost powdery with massed conidia; controls all free. On the fifteenth day a few conidiophores were still visible, but the leaves were all nearly dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from Triticum vulgare Sown on "Cut" Leaves of Hordeum vulgare.
(Experiment No. t1.)

Ten leaves were "cut" and five inoculated. On the seventh day small but vigorous patches of mycelium were formed over the "cuts" on two leaves. On the ninth day three of the five inoculated leaves bore over the "cuts" mycelial patches with young conidiophores; the five control leaves all free. On the thirteenth day the three infected leaves bore little groups of conidiophores with chains of ripe conidia. On the fifteenth day the same; but all the leaves were now nearly dead; controls still free.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from H. vulgare Sown on "Cut" Leaves of Secale cereale.
(Experiment No. p1.)

Six leaves were inoculated. After 6 days two conidia had produced on one leaf short hyphal branches from the appressorium, after penetration of the haustoria, and penetration of several other haustoria had occurred. (What appeared to be failure of the haustorium to develop in the epidermal cell after penetration was observed in several instances.) After 13 days, one small mycelial patch was visible over the "cut" on the one leaf, no conidiophores were observed; all the leaves were now dead.

Conidia Produced on "Cut" Leaves of Triticum dicoccum by Inoculation with Conidia from T. vulgare Sown on "Cut" Leaves of T. dicoccum. (Experiment No. q.)

Three leaves were inoculated. On the second day vigorous infection was evident on two leaves, large lobed haustoria and vigorous mycelial hyphæ being present. On the fifth day the mycelial patches were still growing vigorously, but all the leaves were turned yellow, and nearly dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from H. vulgare Sown on "Cut" Leaves of Triticum vulgare.
(Experiments Nos. d1, f1.)

Eight leaves were inoculated. On four of these the germinating conidia produced

in 7 days fairly vigorous mycelial patches and numerous large lobed haustoria. No conidiophores were observable on the eleventh day, by which time all the leaves were dead.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia from H. vulgare Sown on "Cut" Leaves of H. sylvaticum. (Experiments Nos. *t, v, x.*)

Nine leaves were inoculated, and infection resulted on four. Conidiophores were produced in 4-6 days, and after 14 days small powdery *Oidium*-patches were produced, which persisted, keeping powdery with accumulated conidia, for as long as a month from the date of the sowing of the conidia.

Conidia Produced on "Cut" Leaves of Hordeum sylvaticum by Inoculation with Conidia on "Cut" Leaves of H. sylvaticum Produced by Inoculation with Conidia from H. vulgare Sown on "Cut" Leaves of H. sylvaticum. (Experiments Nos. *c1, q1.*)

Two leaves were inoculated and both became infected. A small but vigorous patch of mycelium, bearing groups of conidiophores, appeared in 10 days, and this became powdery with the massed conidia by the twelfth day. A large continuous powdery *Oidium*-patch, measuring 6 millims. by 3 millims., was produced over the "cut" on one leaf at the end of 4 weeks from the sowing of the conidia.

Bibliography.

1. NEGER, F. W. "Beiträge zur Biologie der *Erysipheen*," 'Flora,' vol. 90, pp. 242-267, 1902.
2. MARCHAL, E. "De la spécialisation du parasitisme chez l'*Erysiphe graminis*," 'Comptes Rendus,' vol. 135, pp. 210-212, 1902.
3. IDEM. *Loc. cit.*, vol. 136, 1280-1281, 1903.
4. SALMON, E. S. "Infection-powers of ascospores in *Erysiphaceæ*," 'Journ. of Bot.,' vol. 41, pp. 159-165, 204-212, 1903.
5. IDEM. "On Specialization of Parasitism in the *Erysiphaceæ*," 'Beihefte z. Botan. Centralbl.,' vol. 14, pp. 261-315, 1903.
6. WARD, H. MARSHALL. "On the Relations between Host and Parasite in the Bromes and their Brown Rust, *Puccinia dispersa* (ERIKSS.)," 'Annals of Botany,' vol. 16, pp. 233-315, 1902.
7. IDEM. "Experiments on the Effect of Mineral Starvation on the Parasitism of the Uredine Fungus, *Puccinia dispersa*, on species of *Bromus*," 'Proc. Roy. Soc.,' vol. 71, p. 151, 1902.

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Phil. Trans., B, vol. 197, 1904, pp. 123-191.

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Rogers, Leonard. Phil. Trans., B, vol. 197, 1904, pp. 123-191.

Colubrine snake-venoms (Naja Bungarus, Bungarus fasciatus, B. caeruleus), action of; antidotal powers of Calmette's antivenin.

Rogers, Leonard. Phil. Trans., B, vol. 197, 1904, pp. 123-191.

Snake-venoms, colubrine and viperine—action and antidotes.

Rogers, Leonard. Phil. Trans., B, vol. 197, 1904, pp. 123-191.

Viperine snake-venoms (Daboia Russellii, African Puff-adder, Cerastes horkelii, Epiwrenurus amabilis) — physiological action of; probable antivenin for.

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VI. The Physiological Action and Antidotes of Colubrine and Viperine Snake Venoms.

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Communicated by Dr. A. D. WALLER, F.R.S.

(From the Physiological Laboratory of the University of London.)

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Part I.—COLUBRINE VENOMS.

ALTHOUGH the poison of the Cobra has long been the subject of careful investigation, those of the other Indian poisonous Colubrine snakes have received comparatively little attention, in spite of their well-known deadliness. Now that CALMETTE's antivenin has been proved to be effective against Cobra venom it is a matter of considerable importance to ascertain if the physiological action of the remaining Colubrine snake-venoms is similar to that of the Cobra or not, and whether CALMETTE's serum is effective against them also, or if it is necessary to prepare others which will act in their case on similar lines. The fact, which I have recently demonstrated (1), that the poison of the Sea-snakes is identical in its physiological action with Cobra venom, although of greater potency, suggests the hope that it may prove possible to obtain a single antidote which will be effective against both these large classes of venomous snakes. I have, therefore, obtained the dried poisons from India, and in Part I. of this paper propose to describe their actions and to discuss the bearing of the facts recorded on the above problem.

I. *Naia Bungarus*, or *Hamadriad*.

This deadly reptile is found, according to FAYRER (2), most frequently in Bengal, Assam, Burma and Southern India, and is by far the largest of the Indian poisonous snakes, and probably the largest in the world, the specimen from which my venom was obtained having measured 13 feet in length. The dried poison has an orange colour. I am not aware that its physiological action has been closely investigated

before, although a few experiments were made with it by FAYRER (2) many years ago, and also I believe by D. D. CUNNINGHAM, who, I am informed, found it to closely resemble Cobra venom.

The symptoms produced by this venom in birds, in a few experiments performed at the Calcutta Zoological Gardens with the kind help of the Superintendent, BABU RAI SANYAL BAHADUR, were found to be precisely similar to those caused by Cobra poison, and the toxicity was also very similar in degree to that of the Cobra, the two being quite indistinguishable from each other by the symptoms produced, as the following example will show.

A pigeon, 225 grammes in weight, was injected with 2 mg. per kilogramme of dried Hamadriad venom, dissolved in 0.5 cub. centim. of normal salt solution, into the breast muscles.

After 30 minutes slight sleepiness and gaping were noted.

„ 40 „ the bird was nodding slightly.

„ 60 „ sleepy, nodding and gaping; respirations very deep and only 20 per minute.

„ 80 „ the same, but cannot stand; respirations 18 per minute.

„ 110 „ the same „ „ „ 16 „

„ 140 „ the same, but cannot raise head off the table, being nearly completely paralysed.

Death took place at the end of $2\frac{1}{2}$ hours. There was no intravascular clotting, and the blood was dark and clotted well on being removed from the vessels. Cobra venom in the same dose produces precisely the same symptoms and proves fatal in about the same time.

Hæmolytic Action.—This has been tested by adding a small drop of blood (5 cub. centims.) to solutions of different strengths of venom in isotonic salt solutions, and counting the number of red corpuscles remaining after varying periods, as well as noting the laking of the fluid and amount of undissolved sediment of corpuscles. It was found that the venom must be used in a solution of the strength of 1 in 1000 to produce marked hæmolysis, and that even with this strength 24 hours at least were necessary at room temperature (14° C.) to produce complete solution of human blood in the proportion of 1 part to 200 of the venom solution. Slight dissolution, however, was observed at the end of 3 hours, and marked after 7 hours. These effects are about the same as those produced by a 1-in-100,000 solution of Cobra venom under similar conditions. It is evident, then, that the hæmolytic action of Hamadriad venom is very small as compared with that of Cobra venom, being in fact about equal to that which I found in the case of the Sea-snake venoms (1), and it can have no direct part in producing the fatal effects of Hamadriad poison.

Blood Pressure and Respiratory Curves.

In order to ascertain how death is produced by Hamadriad venom, tracings of the blood pressure with a Gad manometer and of the respirations with a Sandström recorder connected with a tube in the trachea, have been taken, the poison being injected intravenously. The following table summarises the general results of three experiments:—

TABLE I.

Number.	Animal.	Dose per kilogramme.	Respiration failing.	Blood pressure rising.	Respiration stopped.	Final fall of blood pressure.
		mg.	mins.	mins.	mins.	mins.
	Rabbit	5	1	1½	1½	2
	"	1	6	9½	9½	10
	Cat	2	11	21	20	4½*

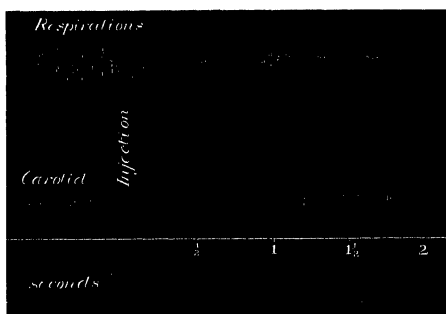
* Artificial respiration performed.

The sequence of events in the first two experiments is the same, the rapidity of the action of the poison being in proportion to the amount injected. The data of these experiments will serve to illustrate the results and bring out some other points of interest.

EXPERIMENT I.—Rabbit, weight $2\frac{1}{2}$ kg., under chloroform followed by ether. Cannula in carotid artery connected with manometer. Tracheal tube inserted and connected with a Sandström recorder. Phrenic nerve exposed on the right side of the neck, and stimulated with induction shocks at intervals, and the contraction of the diaphragm noted. A dose of 11.25 mg. (5 mg. per kilogramme) dissolved in 0.5 cub. centim. normal salt solution was injected into the external jugular vein.

Time.	Blood pressure.	Respirations.		Phrenic nerve.	
		Per ½ min.	Amplitude.	Coil at	Contraction.
	millims.		millims.	millims.	
Before injection	85	45	7	17	Good.
After ½ min.	85	46	7	—	—
" 1 "	85	40	4	—	—
" 1½ mins.	100	18	0	18	Nil.
" 2½ "	40	—	—	15	Slight.
" 4½ "	40	—	—	12	Nil.
" 7 "	25	—	—	5	Good.
				0	Nil.
				—	—

Five minutes later the sciatic nerve responded to stimulation with the secondary coil at 9 millims., and the leg muscles to the same degree, showing that the motor end-plates of these muscles were not completely paralysed at a time those of the phrenics were completely curarised. The blood after death was dark and fluid, but clotted quickly on being placed in a test-tube. No intravascular clotting could be found. (See Tracing I.)



Tracing I. (Hamadriad, Experiment I).—Rabbit. Hamadriad venom. 5 mg. per kilogramme, intravenously.

EXPERIMENT II.—Rabbit, weight 1·7 kg., under chloroform followed by ether. Conditions as in Experiment I, except that the phrenic was not exposed. A dose of 1·7 mg. (1 mg. per kilogramme) in 0·5 cub. centim. salt solution was injected into the external jugular vein.

Time.	Blood pressure.	Respirations.		Remarks.
		Per minute.	Amplitude.	
	millims.		millims.	
Before injection	110	78	8	
After 1 min.	110	79	9	
" 2 mins.	105	81	9	Respiration increased.
" 3 "	103	78	9½	
" 4 "	100	73	9	
" 5 "	100	69	8½	Respiration decreased.
" 6 "	97	62	7½	
" 7 "	97	57	6	
" 8 "	95	51	4	
" 9 "	80	46	2	Blood pressure rising.
" 9½ "	100	6	1	
" 10 "	80	0	—	
" 11 "	10	—	—	
" 12 "	0	—	—	

Post-mortem.—On opening the chest immediately after the final fall of the blood pressure the heart was found to be still beating, confirming the opinion derived from the character of the tracing that the circulatory failure was quite secondary to the cessation of the respiration. There was no clotting in the portal veins, heart or large thoracic vessels, as sometimes occurs in Daboia poisoning. On at once testing the effect of stimulating the nerves with the induced current the results were as follows :—

	Secondary coil at	Contraction of muscle.
Phrenic nerve . . .	0 millims.	Nil.
Diaphragm	10 „	Good.
Sciatic nerve	10 „	Good.
Leg muscles	11 „	Good.

Here, again, we find the motor end-plates of the phrenic nerves completely paralysed immediately after the death of the animal, while those of the leg (and also the arm muscles) were still functionally active. Nevertheless, the data given in the last two columns of the record of Experiment I. show that the phrenic end-plates did not become paralysed until after the respiratory centre had ceased to work, so that (as I also found in the case of the *Enhydrina Bengalensis*), the primary effect of the venom is to paralyse the respiratory centre, the end-plates of the phrenic nerves being affected very shortly afterwards.

Another point of interest is the evidence in the record of Experiment II. of a preliminary stimulus of the respiratory centre by the smaller dose used in this instance, both the number and amplitude of the respirations having been increased during the second and third minutes after the injection of the venom (the effect being clearly observable in the tracing) followed by a slow, but steady, reduction of the breathing until it finally ceased after $9\frac{1}{2}$ minutes, the blood pressure then rising for a brief period before its final fall. These experiments clearly show that the poison of the Hamadriad is identical in its physiological action with that of the Cobra, and, consequently, also with that of the Hydrophidæ. It remained to be ascertained if the failure of the circulation could be averted by the use of artificial respiration as soon as the breathing stopped, as can be done in the case of the last two mentioned venoms, for which purpose Experiment III. was undertaken.

The following experiment shows very well the repeated effect of artificial respiration in lowering the excessive blood pressure due to approaching asphyxia owing to paralysis of the respiratory centre by the Hamadriad venom, just as in the case of *Enhydrina* poison. Further, even after what would naturally have been the final fall of blood pressure had commenced, artificial respiration repeatedly raised it once more, while twice feeble natural respiratory efforts recommenced. Yet within 3 minutes of its being finally left off a fatal fall of pressure occurred. It is evident,

EXPERIMENT III.—A Cat, weighing 3 kgs., was chloroformed and 6 mgs. of Hamadriad venom (2 mgs. per kilogramme) was injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection	125	67	
After 2 mins.	125	71	Respirations quickened.
" 5 "	125	68	
" 8 "	125	63	
" 11 "	125	41	Respirations slowed.
" 14 "	125	31	
" 17 "	115	36	
" 19 "	100	31	
" 20 "	90	6	Respirations ceased.
" 21 "	120	0	Artificial respiration started.
" 22 "	100	0	Artificial respiration left off.
" 23 "	125	0	Artificial respiration re-started.
" 24 "	95	0	Artificial respiration left off.
" 25 "	135	0	
" 27 "	100	28	Feeble natural respiration recommenced.
" 28 "	75	23	Blood pressure falling.
" 29 "	60	5	Respiration ceased.
" 32 "	40	0	Artificial respiration recommenced.
" 35 "	130	—	Blood pressure risen again.
" 36 "	125	—	Artificial respiration left off.
" 38 "	135	17	Feeble natural respiration recommenced.
" 40 "	60	4	Respiration ceased. Blood pressure falling. Artificial respiration resumed.
" 41 "	100	—	Blood pressure risen. Artificial respiration left off.
" 44 "	40	0	Final fall of blood pressure.

then, that by means of artificial respiration cases of poisoning by this venom could be kept alive for long periods, which might conceivably, under certain circumstances, allow of antivenin or other remedies being obtained from some little distance, or of medical aid being brought to the patient, a remark which also applies to Cobra and Sea-snake poisoning.

II.—*Bungarus fasciatus*, or *Banded Krait*.

This snake, according to FAYRER (2) and WALL (3), is very common in Bengal and Eastern and Southern India generally, and grows to a considerable size, but has always been considered less deadly than its smaller congener, *Bungarus caeruleus*, the Krait. Both FAYRER and WALL record a few experiments with it, the former finding that their bites were fatal to dogs in from 4½ hours to 10 days, being much less rapidly fatal than the bites of Cobras, while WALL describes an acute form of poisoning with typical Colubrine symptoms, and also a chronic form, quite different to anything he had met with in the case of other Colubrine snakes, his description

resembling the symptoms of blood poisoning or of the chronic form of Viperine poisoning.

The injection of pigeons throws some light on this peculiarity, for although fairly rapidly fatal subcutaneous doses of the venom cause death with all the usual symptoms of Colubrine poisoning, yet if large doses be given another important change is found *post-mortem*, namely, intravascular clotting, most marked in the portal system, but extending to the pulmonary and systemic circulations. In short, we have to deal with a mixed Colubrine and Viperine poison, just as C. J. MARTIN (4) found in the Australian Colubrine Snake, *Pseudechis porphyracus*. This is further borne out by the antivenin experiments, for it was found that CALMETTE'S antivenin neutralised the Colubrine symptoms, if given in a sufficient dose, but the animals, who had received ten times a fatal dose of *Bungarus fasciatus* venom, all died after 2-4 days of the chronic form of poisoning.

Hæmolytic Action.—This was found to be very feeble in the case of human blood, for when 5 cub. centims. were added to 1 cub. centim. of a 1-in-1000 solution of the venom, hæmolysis was not quite, although very nearly, complete after 24 hours. It is thus as feeble as Hamadriad and Enhydrina venoms in this respect.

Blood Pressure and Respiratory Curves.

The following table summarises four experiments in which blood pressure and respiratory tracings were taken after intravenous injection of this poison :—

TABLE II.

Number.	Animal.	Dose per kilogramme.	Respiration falling.	Blood pressure rising.	Respiration ceased.	Final fall of blood pressure.
		mg.	mins.	mins.	mins.	mins.
1	Rabbit . .	5	7	12	9	15
2	" . .	10	3	3	4	4½
3	" . .	20	2	2½	4	5
4	Cat . . .	10	8	Nil	19	21*

* Artificial respiration carried on.

Here, again, we see the same sequence of events as in the case of the Hamadriad, but a different degree of toxicity. Thus, 5 mg. per kilogramme of the latter killed more quickly than 10 mg. of the *Bungarus fasciatus* poison did, while 5 mg. of the latter was about as deadly as 1 mg. of the Hamadriad venom. Nevertheless, the following data of the experiments prove that the physiological action of the two is identical in its principal lethal action and similar to that of Cobra venom, but with the addition of another factor :—

EXPERIMENT I.—Rabbit, 1·8 kg., under chloroform followed by ether. Conditions the same as in the Hamadriad experiments above. A dose of 9 mg. (5 mg. per kilogramme) in 9 cub. centim. normal salt solution injected intravenously

Time.	Blood pressure.	Respirations.		Remarks.
		Per minute.	Amplitude.	
	millims.		millims.	
Before injection . . .	100	70	7	
After 1 min.	85	68	7½	Fall of blood pressure.
" 2 mins.	65	72	8	
" 3 "	60	75	8	Respirations quickened.
" 4 "	75	76	8	
" 5 "	80	75	7	
" 6 "	85	69	6	
" 7 "	80	62	4½	Respirations slowed.
" 8 "	80	58	3	
" 9 "	80	53	1½	
" 10 "	80	34	1	
" 11 "	80	24	½	Respirations stopped.
" 12 "	100	0	—	
" 13 "	110	—	—	Respiratory convulsions.
" 14 "	110	—	—	
" 15 "	55	—	—	
" 17 "	25	—	—	

Post-mortem, the portal vein was found to be distended with fluid blood together with a soft clot, which is of interest in connection with the marked primary fall of blood pressure during the second and third minutes. The heart, pulmonary, arterial, and other large thoracic vessels were free from clot. The result of testing the nerves and muscles with the induced current was as follows :—

	Secondary coil at	Contraction of muscle.
Phrenic nerve	0 millims.	Nil.
Diaphragm	10 "	Good.
Sciatic nerve and brachial plexus	11 "	Good.
Leg and arm muscles	11 "	Good.

Here, once more, we find the motor end-plates of the phrenic nerves completely paralysed, while the limb nerve trunks are still physiologically active.

ANTIDOTES OF COLUBRINE AND VIPERINE SNAKE VENOMS.

EXPERIMENT III.—Rabbit, weight 1.15 kg., under chloroform followed by ether.

Conditions the same as in the above experiment, but a dose of 23 mg. (20 mg. per kilogramme) in 1 cub. centim. salt solution was given intravenously.

Time.	Blood pressure.	Respirations.		Remarks.
		Per $\frac{1}{2}$ min.	Amplitude.	
	millims.		millims.	
Before injection . .	100	52	7	
After $\frac{1}{2}$ min. . .	75	54	$7\frac{1}{2}$	Primary blood pressure fall.
" 1 " . . .	65	56	$7\frac{1}{2}$	
" $1\frac{1}{2}$ mins. . .	60	52	6	
" 2 " . . .	65	45	3	Respiration failing. Blood pressure rising.
" $2\frac{1}{2}$ " . . .	115	35	2	
" 3 " . . .	120	$2\frac{1}{2}$	1	
" $3\frac{1}{2}$ " . . .	115	19	1	Respiration ceased. Blood pressure falling.
" 4 " . . .	120	8	$\frac{1}{2}$	
" 5 " . . .	75	—	—	
" 7 " . . .	50	—	—	
" 9 " . . .	20	—	—	

Post-mortem, the heart was found to be beating, and continued to do so for 15 minutes. There was no clotting of the blood in the portal vein, the heart, or the large thoracic vessels. The reactions of the nerves and muscles to the induced current were as follows :—

	Secondary coil at	Contraction of muscle.
Phrenic nerve	0 millim.	Nil.
Diaphragm	5 "	Good.
Sciatic and brachial nerves . .	0 "	Nil.
Leg and arm muscles	9 "	Good.

It is interesting to observe that, with the large and rapidly fatal dose of poison given in this case, the motor end-plates of the limb muscles, as well as of the diaphragm, were paralysed completely at the time of death, just as I had previously found to be the case when large doses of Enhydrina poison were injected. The absence of any clotting in the portal vein with this large dose, as well as in Experiment II. in which 10 mg. per kilogramme was injected, shows that its occurrence in the first experiment was of an exceptional nature. On the other hand, all three tracings show a marked primary fall of pressure occurring within 1 or 2 minutes of injection. This feature I have also found in a much smaller degree and somewhat inconstantly in my experiments with Enhydrina and Cobra poisons (although not with Hamadriad venom), but it is much more marked and constant in the case of the *Bungarus fuscatus* venom, which is a less powerful poison, weight for weight, to

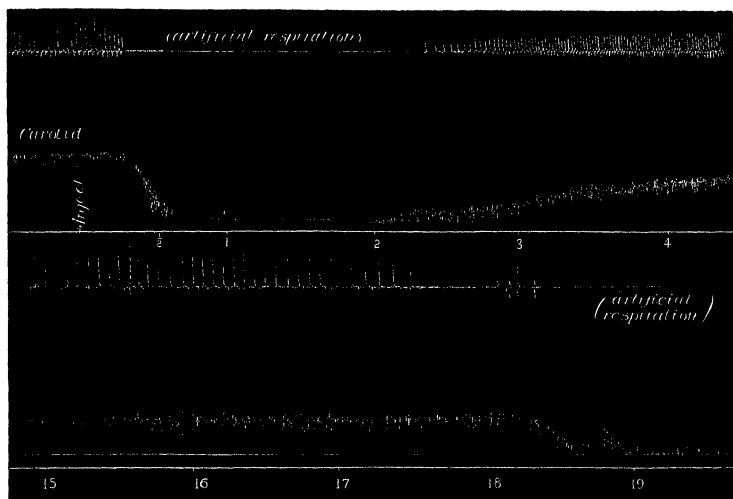
the respiratory centre than the other two. This blood-pressure fall appeared to be similar in nature to that which I have found to be so marked and essential a feature of poisoning by the Vipers and Pit Vipers (as shown in Part II. of this communication), and is, doubtless, due to the presence of a small quantity of the principal constituent of the Viperine poisons. In order to examine the effect of this venom on the blood pressure further, the following experiments were done on cats, and at the same time the effect of artificial respiration in keeping the circulation going after natural respiration had ceased was tested.

EXPERIMENT IV.—A Cat, weighing $3\frac{1}{2}$ kg., was given chloroform followed by ether, and 3.5 in 1 cub. centim. salt solution (1 cg. per kilogramme) injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection .	140	31	
After $\frac{1}{2}$ min. . .	70	9	Blood pressure fallen. Respirations stopped.
" 1 " . .	50	0	Artificial respiration started.
" 2 mins. . .	50	0	
" 3 " . .	65	19	Respirations recommenced.
" 4 " . .	80	49	Blood pressure rising.
" 6 " . .	90	55	
" 7 " . .	90	49	
" 8 " . .	95	37	Respirations slowing.
" 10 " . .	95	27	
" 13 " . .	85	20	
" 16 " . .	70	14	
" 19 " . .	20	1	Respirations ceased. Artificial respiration.
" 21 " . .	0	0	Respirations ceased. Artificial respiration.

(See Tracing II., 1-4 and 15-19 minutes.)

In this case we have a very marked primary fall of blood pressure, which was rapidly recovered from to a very large extent, followed by the typical steady failure of respiration of Colubrine poisons, while artificial respiration after the cessation of natural breathing failed to keep the circulation going as in the case of the other Colubrine poisons. The circulatory conditions were very similar to those which I have found to occur constantly in the case of Viperine poisons, so this experiment confirms my conclusion that the poison of the Banded Krait is a mixture of both Colubrine and Viperine poisons, just as C. J. MARTIN showed the Colubrine *Pseudechis* of Australia to be. As the Viperine poisons are more readily destroyed by heat than is the Colubrine element, and in the case of the *Pseudechis*, C. J. MARTIN (4) has shown that the Viperine action can be eliminated by heating the venom to 90° C. for a very short time, leaving the Colubrine action intact; the following experiment



Tracing II. (*Bungarus fasciatus*, Experiment IV.).—Cat. *B. fasciatus* venom. 10 mg. per kilogramme, intravenously.

was performed to see if the same occurred in the case of the venom of the *Bungarus fasciatus*.

EXPERIMENT V.—A Cat, weighing $3\frac{1}{2}$ kg., was given chloroform followed by ether, and 3.5 cc. (10 mg. per kilogramme) of the venom of *Bungarus fasciatus*, which had previously been heated in solution in 0.5 cub. centim. normal salt solution to a temperature of 90° C. for $\frac{1}{2}$ minute in a water-bath, was injected intravenously.

Time.	Blood pressure.	Respirations per $\frac{1}{2}$ minute.	Remarks.
Before injection . . .	millims. 135	17	
After $\frac{1}{2}$ min.	100	20	
" 1 "	60	8	Respirations stopped.
" $1\frac{1}{2}$ mins.	75	15	Respirations recommenced.
" 2 "	90	10	
" $2\frac{1}{2}$ "	95	8	
" 3 "	95	6	
" $3\frac{1}{2}$ "	95	1	Respirations stopped.
" 4 "	60	0	Blood pressure falling. Artificial respiration begun.
" 5 "	90	0	
" 7 "	70	—	Artificial respiration left off.
" 8 "	55	—	

The pressure rose again on recommencing artificial respiration, and was kept up by its means until the 15th minute, but on then leaving it off an asphyxial rise followed rapidly by a final fall occurred. After death the phrenic nerves were found to be partially paralysed, while the blood collected immediately after death in a test-tube clotted firmly, and the next day a quantity of clear serum had exuded, showing that no marked hæmolysis had resulted from the action of the poison.

It is evident from the above data that the raising of the temperature of a solution of the venom to 90° C. for a very short time—opalescence resulting from the coagulation of some albuminous body present—had the effect of greatly lessening the action of the poison in causing a fall of blood pressure, while it did not reduce its action on the respiratory centre. It is also worthy of note that in this experiment artificial respiration had a much more marked effect in maintaining the blood pressure than in Experiment IV, with unheated venom. These facts confirm the supposition that the venom of the *Bungarus fasciatus* contains a mixture of Viperine and Colubrine poisons, the former being much more readily destroyed by heat, and thus closely resembles the venom of the *Pseudechis* in its general characters. It differs, however, from the last named in not having any powerful paralysing action on the heart, for I found that even a solution of the strength of 1 in 50 produced no effect on the beat of a frog's heart to which it was directly applied, while MARTIN (4) found *Pseudechis* venom to stop the frog's heart in diastole when applied in strong solution.

As LAMB (5) has shown that *Daboia* venom in a weak solution is completely destroyed by a temperature of 73–75° C. for $\frac{1}{2}$ hour, while Cobra venom is little if at all affected by such treatment, I treated some *Bungarus fasciatus* venom in this way, and in Experiment VI. injected a cat under chloroform intravenously with the same dose as in the previous cases (10 mg. per kilogramme), but at the end of 1 hour it had produced no effect on either the blood pressure or the respiratory rate. This fact is of considerable interest, for the main action of the venom is undoubtedly of the Colubrine type causing paralysis of respiration, and the symptoms produced on birds, rats and mice are precisely similar to those of Cobra venom itself, yet the poison is rendered inert by heating to 73–75° C., and in the case of mice twenty times a minimal lethal dose thus heated proved harmless. It is evident then that LAMB and HANNA'S (5) contention that *Daboia* venom contains no element of the Colubrine type is not proved by his experiments showing an absolute loss of virulence by heating to 73–75° C., for there might be such an element present which is destroyed by that temperature; as in the case of the Banded Krait venom.

III.—*Bungarus cœruleus*, or *Krait*.

This is one of the commonest and most dreaded of the poisonous Indian snakes, being very deadly in spite of its comparatively small size as compared with the Cobra, &c., and on account of its wide distribution it is the cause of a large amount of mortality. Thanks to the kindness of Dr. V. W. SHAW in giving me a few milligrammes of the venom of this snake, I have been able to get one tracing and to do some other experiments with its poison. It is slightly more virulent than Cobra venom, the minimal lethal dose for pigeons being 0.25 mg. per kilogramme, while the symptoms it produces are identical with those of the Cobra, as pointed out by FAYRER (2), and WALL (3), with whose observations on this point my own agree.

EXPERIMENT I.—Rabbit, weighing $1\frac{1}{2}$ kg., under chloroform followed by ether.

Injected intravenously with 8 mg. of Krait venom (4 mg. per kilogramme). The phrenic nerve was exposed in the neck and stimulated with induction shocks at intervals of 1 minute.

Time.	Blood pressure.	Respirations.		Phrenic nerve.	
		Per minute.	Amplitude.	Coil at	Contraction
	millims.		millims.	millims.	
Injection. . .	60	63	5	30	Good.
1 min. . .	40	61	5	30	Good.
2 mins. . .	40	56	3	30	Good.
3 " . .	40	50	2	30	Fairly well.
4 " . .	80	21	1	30	Feebly.
5 " . .	30	0	—	30	Nil.
				25	Fairly well.
6 " . .	20	—	—	25	Weaker.
7 " . .	20	—	—	25	Feebly.
8 " . .	15	—	—	20	Fairly well.
9 " . .	10	—	—	—	

Here we have the typical paralysis of the respiratory centre, quickly followed by partial paralysis of the phrenic end-plates. In birds after death from this poison the sciatic nerves were also paralysed, just as in Cobra poisoning. The blood pressure showed a slight fall, followed by a marked rise with the advancing respiratory failure and a final fall after complete cessation of respiration. Artificial respiration was not tried, but there can be little doubt that it would be as efficacious in Krait poisoning as in that due to Cobra or Hamadriad and Enhydrina venoms. It is noteworthy that the end-plates of the phrenic nerves were not paralysed completely at the time death occurred, this effect being somewhat less than with the Cobra, Hamadriad and Enhydrina venoms, so that in the case of the Krait it is still more clear that the respiratory failure occurs before that of the phrenic end-plates.

Hæmolytic Action.—In the case of pigeon's blood, when 5 cub. centims. was added to 1 cub. centim. of a 1-in-10,000 solution of Krait venom in 0.9 salt solution complete hæmolysis took place within 6 hours. Similarly, with a 1-in-100,000 solution three-fourths of the red corpuscles were dissolved in 24 hours, while after 2 days it was nearly complete. With human blood the effect was not quite so great, as when the same quantity of blood was added to a 1 cub. centim. of a 1-in-10,000 solution the hæmolysis was not quite complete after 24 hours, although it was nearly so.

If we compare these data with those obtained in the same way for other snake venoms we find the hæmolytic action of Krait venom to be much less than that of Cobra poison; but, on the other hand, it is somewhat greater than that of Hamadriad or Enhydryna venoms, and also than that of the Banded Krait, being intermediate between these last three and Cobra venom.

Conclusions as to the Actions of the Indian Colubrine Poisons.

The physiological action of the three Colubrine Snake poisons dealt with, namely, the Hamadriad, Banded Krait and Common Krait, can be briefly summarised as follows:—

I. *Action on the Respiratory Centre.*—The essential lethal action of all these venoms, like that of the Cobra and the Hydrophidic, is one of paralysing the respiratory centre, the respirations becoming both fewer in number and less in amplitude minute by minute until they cease when a large dose of the poison is administered intravenously. With smaller doses a very temporary stimulation of the respiratory centre is produced first.

II. *Action on the Motor End-Plates of the Phrenic and other Nerves.*—Next in importance is the paralysis of the end-plates of the phrenic nerves producing paralysis of the diaphragm, which follows very closely on the failure of the respiratory centre. This effect appears to be somewhat less marked in the case of the Krait venom than with the other two. If a large and rapidly fatal dose is given then the end-plates of the sciatic nerves are also paralysed before death occurs, but with smaller doses they usually only show partial paralysis at a time when the phrenic nerves are completely paralysed.

III. *Hæmolytic Action.*—The power of these venoms in dissolving the red corpuscles is very much less than that of Cobra venom, being about one hundred times less powerful in the case of the Hamadriad and Banded Krait, and about ten times less so in that of the Common Krait. Yet these venoms produce precisely the same symptoms as Cobra poison, so that the lethal effects of the latter cannot be in any degree due to its hæmolytic action, which becomes a very secondary property of Colubrine venoms in view of these results.

IV. *Action on the Blood Pressure.*—The blood pressure always rises during the asphyxial stage following the cessation of respiration, and subsequently falls again at

the time of death. The circulation, however, can be kept going for a long time after complete failure of the breathing if artificial respiration is kept up in the case of the Hamadriad, as well as in the Cobra, as first shown by LAUDER BRUNTON and FAYRER (6). In the case of *Bungarus fasciatus*, however, this is not the case, and in this instance marked primary fall of blood pressure occurs very shortly after intravenous injections of the venom. This we have seen is due to an added Viperine element, which may cause intravascular clotting in the portal system and in small animals throughout the body. This primary fall of pressure is similar to those always produced by Viperine poisons, and may be greatly lessened by heating the venom to 90° C. for a short time. In the case of Cobra and Krait venoms also slight primary falls of blood pressure are met with, which may possibly also be due to very minute amounts of the Viperine element in these venoms. It is only in the case of *Bungarus fasciatus* that this primary fall of pressure is of much importance.

The Relative Antidotal powers of Antivenin (CALMETTE'S), prepared mainly by the injection of Cobra Venom, against the Poisons of the Hydrophidæ and Indian Colubrine Snakes.

The facts recorded in the first part of this paper, and in a previous one (1), show that the physiological action of the Hydrophidæ, the Hamadriad and the Krait, as well as the most important element in the venom of the Banded Krait (*Bungarus fasciatus*), are all similar to that of the Cobra. Further, we have in CALMETTE'S antivenin an efficient antidote to the venom of the Cobra when it is given in sufficient doses, this serum being obtained from horses which have been actively immunised to a very high degree by frequent injections of a mixture of venoms which is mainly composed of Cobra poison. It becomes, then, a matter of great practical importance to ascertain whether CALMETTE'S antivenin is efficient against the other venoms mentioned, and if so, in what doses it must be used for curative purposes. Through the kindness of M. CALMETTE in placing at my disposal the necessary serum, I have been able to make a series of experiments on this point with results of some interest. The method adopted was that recommended by C. J. MARTIN (7), namely, the mixing at least ten minimal lethal doses of the poison with given quantities of the serum and leaving them in contact for at least $\frac{1}{2}$ hour at room temperature (about 18–20° C.), before injecting them subcutaneously, pigeons and rats being used for the experiments.

The first thing was to test the action of the antidote against Cobra venom, so as to standardise it and to get a term for comparison with other venoms. But there are two common varieties of Cobras in Bengal, one of which is much more frequently met with than the other, and consequently its venom was mainly used for the production of antivenin. As I had poison from these two kinds separately collected by Assistant-Surgeon DALY, in Calcutta, I have tested each of them with the following

results. The common variety is called by the natives the Keautiah, and the rarer one the Gokurrah, the former having one ocellus, or rounded mark, on the hood, and the latter having a double ocellus resembling a pair of spectacles.

I. *Cobra (Keautiah).*

The minimal lethal dose per kilogramme weight of the poison used was 0.5 mg. Rats of about 150 grammes in weight were used, the minimal lethal dose for which would be 0.075 mg., so as ten minimal lethal doses were to be given, each received 0.75 mg. of the dried venom, which had been dissolved in a fraction of a cubic centimetre of normal salt solution and added to varying quantities of the serum to be tested $\frac{1}{2}$ hour before injection at room temperature (18–20° C). The results are shown in the following table :—

TABLE III.

Number.	Animal.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
1.	Rat . .	150	0.75	10	0.5	Died in 2½ hours.
2	" . .	150	0.75	10	1.0	Died in night.
3	" . .	150	0.75	10	1.2	Recovered.
4	" . .	150	0.75	10	1.4	Recovered.
5	" . .	150	0.75	10	1.5	Recovered.

As recovery would take place if just over nine of the ten minimal lethal doses had been neutralised (the remaining quantity being then less than a minimal lethal dose), we see that 1.2 cub. centims. of the serum neutralised 0.675 cub. centim. of the venom (0.75–0.075 mg.). One cub. centim. would, therefore, neutralise 0.5625 mg., so that it would take 17.7 cub. centims. to neutralise 10 mg. of the venom. LAMB and HANNA (5) had obtained a very similar result, as they found 1 cub. centim. fresh CALMETTE's antivenin neutralised 0.6–0.7 mg. of dried Cobra venom.

II. *Cobra (Gokurrah).*

In a similar manner the action of the antivenin was tested against the poison of the other variety of Cobra, the same quantity being used as in the above, namely, 0.75 mg. in each experiment, although the minimal lethal dose of this poison worked out at 0.4 mg. per kilogramme. The number of minimal lethal doses given, therefore, was 12.5. The results are shown in Table IV.

TABLE IV.

Number.	Animal.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
1	Rat . .	150	0·75	12·5	0·5	Died in 1½ hours.
2	" . .	150	0·75	12·5	1·0	Died in 1½ hours.
3	" . .	150	0·75	12·5	1·5	Died in 2 hours.
4	" . .	150	0·75	12·5	2·0	Died in the night.
5	" . .	150	0·75	12·5	2·5	Recovered.
6	" . .	150	0·75	12·5	3·0	Recovered.
7	" . .	150	0·75	12·5	3·5	Recovered.

In this case we find that 2·5 cub. centims. of the serum neutralised 0·69 cub. centim. (0·75–0·06 mg.) of the poison. One cub. centim. would, therefore, neutralise 0·276 mg., so that it would require 36·2. cub. centims. to neutralise 10 mg. of this venom, or twice as much as in the case of the former variety of Cobra, a difference which is only in a slight degree accounted for by the somewhat greater virulence of the latter kind, as shown by its lower minimal lethal dose. This is a remarkable point, which will be returned to after the tables for the other venoms have been given.

III. Naia Bungarus, or *Hamadriac*.

The minimal lethal dose of this venom worked out at 0·6 mg. per kilogramme weight, while as pigeons of about approximately 250 grammes were used in the experiments (on account of a difficulty in obtaining white rats) the minimal lethal dose was 0·15 mg., and ten doses equalled 1·5 mg. of venom.

TABLE V.

Number.	Animal.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
1	Pigeon .	250	1·5	10	1	Died in 1 hour.
2	" .	250	1·5	10	2	Died in the night.
3	" .	250	1·5	10	3	Died in the night.
4	" .	250	1·5	10	4	Recovered.
5	" .	250	1·5	10	5	Recovered.
6	" .	250	1·5	10	6	Recovered.

Here we find that 4 cub. centims. of serum neutralised 1·35 mg. (1·5–0·15) of the *Hamadriac* venom, so that 1 cub. centim. would neutralise 0·3475 mg., and it would require 28·8 cub. centims. of the serum to neutralise 10 mg. of the poison. It

is very interesting to observe that the antivenin made by injecting Cobra venom has an undoubted effect in neutralising the venom of a different kind of snake, although a nearly allied one, in only a slightly less degree than in the case of one variety of Cobra, namely, that called by the natives the Gokurrah.

IV. *Bungarus cœruleus*, or *Krait*.

The minimal lethal dose of this poison was found to be 0·25 mg. per kilogramme, being thus stronger than Cobra venom, although not so strong as the Sea-snake *Enhydrina Bengalensis*. Rats of approximately 80 grammes in weight were used, the minimal lethal dose for which would be 0·02 mg., and 0·2 mg. or ten minimal lethal doses were injected after mixing with the serum as usual.

TABLE VI.

Number.	Animals.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
1	Rat . .	80	0·2	10	1	Died in 7 hours.
2	" . .	80	0·2	10	2	Died in the night.
3	" . .	80	0·2	10	2·5	Recovered.
4	" . .	80	0·2	10	3·0	Recovered.
5	" . .	80	0·2	10	5	Died in two days.*

* Great inflammatory changes at seat of injection, no symptoms of Colubrine poisoning observed. Death due to septic changes.

In this instance it took 2·5 cub. centims. of the serum to neutralise 0·18 mg. (0·2-0·02) of Krait venom. One cub. centim. would therefore neutralise 0·072 mg., and it would take 138·8 cub. centims. of the serum to neutralise 10 mg. As the strength of the venom was twice as great as that of the Keautiah venom, this amount of serum would have to be halved when comparing the effect of the serum on these two poisons. Even then the serum will be about four times as effective against the Cobra venom as against that of the Krait.

V. *Bungarus fasciatus*, or *Banded Krait*.

We have seen that FAYRER (2) pointed out years ago that this snake, although a large one, is much less deadly than the Cobra, and WALL came to a similar conclusion (3), but I am not aware that its exact minimal lethal dose has been accurately worked out. The following table shows the results of inoculating pigeons with varying doses of the venom :—

TABLE VII.

Number.	Animal.	Dose per kilogramme.	Result.
		mg.	
1	Pigeon . .	1	Recovered. No symptoms.
2	" . .	2	Recovered. No symptoms.
3	" . .	3	Recovered. Slight symptoms.
4	" . .	5	Recovered. Slight symptoms.
5	" . .	6	Recovered. Marked symptoms.
6	" . .	7	Died in 8 hours.
7	" . .	8	Died in 1 hour.

Thus we find that the minimal lethal dose of this venom for birds is 7 mg. per kilogramme, or fourteen times as great as that of Cobra venom, and twenty-eight times as great as that of its near relation *Bungarus cœruleus*, or ordinary Krait, and no less than 140 times as great as that of the Enhydryna. This explains the generally acknowledged lesser degree of virulence of the Banded Krait. Such great differences in the toxicity of the venoms of different Colubrine and Sea-snakes, the physiological action of which are identical (with the exception of the added Viperine element in the case of *Bungarus fasciatus*, which probably accounts for the much smaller proportion of the Colubrine element in its venom), are presumably due to varying proportions of the active respiratory poisons in their compositions; a factor which must be taken into account in estimating the amount of antivenin which is required to neutralise the effects of any given snake, together with the amount of the poison ejected by it at a single bite. The markedly small virulence of the poison of *B. fasciatus* as compared with that of the other Indian Colubrine snakes is of great interest in connection with the presence of a Viperine element in this venom, as I have shown in the first part of this paper; for this element, although less toxic than the Colubrine portion, nevertheless is of some bulk, as shown by the marked coagulum produced by heating for a few moments to 90° C., which we have seen above reduces considerably the effect of the Viperine element without affecting the Colubrine portion. This fact no doubt accounts to some extent for the greater minimal lethal dose of this poison.

The experiments on the action of the serum on this poison were carried out with rats, but owing to the small amount of venom in hand, only 3 mg. were used for each rat, of approximately 60 grammes, which amounted to seven minimal lethal doses. The results are shown in the following table:—

TABLE VIII.

Number.	Animal.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
1	Rat. . .	60	3	7	1	Died in night. Ill after 2 hours.
2	" " "	60	3	7	1.5	Died in night.
3	" " "	60	3	7	2	Died after 3 days.
4	" " "	60	3	7	3	Died after 4 days.
5	" " "	60	3	7	5	Died after 4 days.

The first two animals died with the typical symptoms of Colubrine poisoning, but the remaining three died with signs of chronic Viperine poisoning only, the Colubrine element alone having been neutralised by the antivenin.

VI. *Enhydrina Bengalensis*.

The very virulent poison of this Sea-snake I have shown in a previous paper (1) to have identically the same physiological action as Cobra venom. It therefore becomes a matter of interest and practical importance to ascertain whether CALMETTE'S serum has any effect against it. The following series of experiments were carried out in the same way as the foregoing, pigeons of approximately 300 grammes in weight being used, and ten minimal lethal doses being injected. The minimal lethal dose of this virus is 0.05 mg. per kilogramme, or one-tenth of that of Cobra venom, and one-fifth of that of Krait venom, the strongest of the Indian Colubrine snake venoms. A pigeon injected with this dose as a control to the following series with serum added to the virus, died in $2\frac{1}{2}$ hours :—

TABLE IX.

Number.	Animal.	Weight.	Dose.	Minimal lethal doses.	Serum.	Result.
		grammes.	mg.		cub. centims.	
	pigeon . .	300	0.15	10	1	Died in $1\frac{1}{2}$ hours.
	" " "	300	0.15	10	2	Died in $6\frac{1}{2}$ hours.
	" " "	300	0.15	10	3	Died in 30 hours.
	" " "	300	0.15	10	4	Died in 32 to 40 hours.
	" " "	300	0.15	10	5	Died in the night.
	" " "	300	0.15	10	6	Recovered.
	" " "	300	0.15	10	7	Recovered.

Here we find that, although smaller doses caused great retardation of the onset of the symptoms and prolongation of life, yet 6 cub. centims. of serum were required

to neutralise 0·135 mg. of the poison (0·15–0·015), so that 1 cub. centim would neutralise 0·025 mg., while 444 cub. centims. would be required to neutralise 10 mg., which I have found to be the average quantity ejected at one bite by this Sea-snake. The venom of this snake, however, is ten times as powerful, weight for weight, as Cobra venom, presumably owing to its containing ten times as large a proportion of the toxic principle, so that in order to compare the action of the serum on the two it will be necessary to divide the figure just given by ten, which gives 44 cub. centims. for the Enhydrina venom against 17·7 and 28·9 cub. centims. respectively for the two varieties of Cobra venoms. Considering the Enhydrina belongs to a different class of poisonous snakes to the Cobras, it is somewhat remarkable that the antivenin prepared from Cobra venom should have so marked an effect on it, and this fact agrees with the results I have obtained showing the physiological action of the Colubrine and the Sea-snakes is identical. In a few unsuccessful experiments with the antivenin in Calcutta only 0·5 cub. centim. of serum was used (1).

The Relative Value of CALMETTE'S Serum against the Venoms of the Hydrophidæ and Indian Colubrine Snakes.

We now have the necessary data for comparing the action of CALMETTE'S antivenin on the venoms of the Enhydrina and the Indian Colubrine snakes, for the above six series of experiments were carried out with the same serum of the same date. In order to know how much serum would be required to neutralise a natural dose of virus in a man we require to know how much venom different kinds of snakes inject at a single bite. Again, in the above experiments the serum was mixed with the venom for $\frac{1}{2}$ hour before injection, but it would require a larger quantity to counteract a given amount of venom when it is injected after the bite, how much more could only be found out by experiments, which I have not yet been able to undertake. The data, as far as they are available, are embodied in Table X. (p. 147). The first column shows the minimal lethal dose, per kilogramme weight for a warm-blooded animal, of each poison; while the second shows the number of cubic centimetres of serum required to neutralise *in vitro* 10 mg. of each venom. As, however, some of the poisons contain very different proportions of the respiratory poison, as shown by their greatly different minimal lethal doses, this factor must be taken into account in estimating the action of the antitoxin on the actual toxin of the venoms. Thus the poison of *Bungarus fasciatus* has a minimal lethal dose which is fourteen times as great as the common form of Cobra (the Keautiah), so that it must contain only one-fourteenth of the toxin that is present in an equal weight of Cobra venom; and in order to compare the action of the serum on the toxin of each, it will be necessary to multiply by fourteen the amount which neutralises 10 mg. of *B. fasciatus* poison in order to make the figure comparable with that found for the Cobra venom.

Similarly, as the venom of the Enhydrina is ten times as powerful as that of the Cobra, the amount of serum which will neutralise 10 mg. of this venom must be divided by ten before it can be compared with the quantity required to neutralise 10 mg. of Cobra venom. Similarly, the figure for Krait venom must be divided by two, and so on with the others. In this way the figures of Column 3 have been arrived at, to show the relative action of the antitoxin on the equivalent quantities of the active respiratory paralysing toxin of the different venoms.

Next, we must know how much of the serum is necessary to neutralise a minimal lethal dose of the venoms for a man of, let us say, 50 kilos. weight (about the average for natives of India). In the case of the Cobra, with a minimal lethal dose of 0.5 mg. per kilogramme this will be 25 mg., so if we multiply the figures in Column 3, which represents the serum to neutralise 10 mg., by two and a-half, we shall obtain the required results. Lastly, we must know the number of minimal lethal doses for a man of 50 kilos. ejected by the different snakes at a single bite, in order to be able to estimate the amount of serum required to completely neutralise this amount of venom *in vitro*.

In the case of the Cobra, LAMB (8) quotes CALMETTE as giving the average amount of venom ejected by a Cobra at 0.03-0.045 gramme, but, on the other hand, D. D. CUNNINGHAM gives the amount at the much higher figure of 0.254 gramme of dried venom, which would amount to ten minimal lethal doses for a man of 50 kilos., requiring over 400 cub. centims. of serum to neutralise it *in vitro*, and correspondingly more if injected after the poison. Again, I found the average amount of venom to be obtained from an Enhydrina at a single bite to be 10 mg., with a toxicity equivalent to 100 mg. of Cobra venom, or four minimal lethal doses. To completely neutralise *in vitro* this amount (for in the case of man it would not be advisable to be content with reducing the virus to just below a minimal lethal dose) 444 cub. centims. of the serum would be necessary. The Hamadriad, being a much larger snake than the Cobra, would presumably eject a much larger dose of venom, but exact figures are not available as far as I know. In a single specimen, from which my poison was obtained, the amount got was not much larger than a Cobra ejects, but it would not be safe to draw any conclusions from this solitary observation. As, moreover, the serum is only half as active against this venom as against that of the common form of Cobra, it is evident that something like 1000 cub. centims. of the serum would be required to neutralise a natural dose of Hamadriad venom.

In the case of *Bungarus caruleus*, or common Krait, the dose ejected is much smaller than in the case of the Cobra; but this is largely compensated by its greater toxicity, while the serum has a very feeble action against this poison as compared with Cobra venom, so that several hundred cubic centimetres would be necessary to neutralise *in vitro* a natural dose of this venom. In the case of

B. fasciatus, or Banded Krait, the large size of the snake is compensated by the small toxicity of the venom; but, on the other hand, the serum has only a slight action against the respiratory paralysing toxic body present, while it has no action against the Viperine element, or the tendency for septic conditions to ensue, probably due to a loss of bactericidal properties of the blood (a point which I hope to examine shortly). On the whole, although the serum is well worthy of use in these cases, especially as the dose ejected is often only slightly above a minimal lethal one, yet too much must not be expected from its action.

The data summarised in Table IX., then, are somewhat disappointing, in so far as they point to the present available serum being not strong enough to be of very great value in the case of poisoning by the Hydrophidæ and Indian Colubrine snakes when a full dose has been injected by them, yet it is encouraging to find that it has a very definite action against the venoms of all of them, which will make it of service in large doses, especially where only a slightly *supra*-minimal lethal dose may have been injected, while if the serums could be made stronger than they are at present they would be of inestimable service in the treatment of these classes of snake bites. I would suggest that a mixture of Colubrine poisons should be used in the preparation of the antivenom as likely to yield a more generally useful serum.

On the other hand, in the case of the Viperine poisons my experience agrees with that of others in showing that CALMETTE'S serum has no action whatever in neutralising them, and it will be necessary to prepare a separate serum for use against the Vipers.

TABLE X.

Snake.	Minimal lethal dose in mg.	CALMETTE'S Serum to neutralise 10 mg.	Serum to neutralise toxine equivalent to 10 mg. of Cobra.	Serum to neutralise one lethal dose for 50 kg. in man.	Grammes of venom ejected at one bite.	Lethal doses ejected at one bite.	Serum to neutralise amount ejected at one bite <i>in vitro</i> .
1. Cobra (Keautiah) . .	0.5	17.7	17.7	44.25	0.030* 0.254†	10	cnb. centims. 442
2. Cobra (Gokurrah) . .	0.4	36.2	28.9	72.25	0.254	10	722
3. Hamadriad	0.6	28.8	34.5	86.25	?	10	862
4. <i>Bungarus ceruleus</i> . .	0.25	166.0	83.0	207.5	?	(3) †	(600) †
5. <i>Bungarus fasciatus</i> . .	7.0	No effect.	—	—	?	—	—
6. <i>Enhydrina</i> (Sea-snake)	0.05	444.4	44.4	111.0	0.010	4	444

* D. D. CUNNINGHAM.

† LAMB, quoting CALMETTE.

Part II.—VIPERS AND PIT VIPERS.

A. VIPERIDÆ.

During recent years a great deal of work has been done on the action of Viperine poisons on the blood, and their lethal action has mainly been attributed to such action. Thus, in the case of the Rattlesnake WEIR MITCHELL and REICHERT (9) long ago described the loss of coagulability and hæmorrhages which form the most marked features of poisoning by the American Pit Vipers, although they also attributed the lethal effect in part to the paralysis of the respiratory centre and the heart. FLEXNER (10), who more recently has closely examined the blood changes, has supported these views, and described separate toxic bodies, which he calls hæmorrhagin, hæmolysin, and neurotoxin respectively; the latter being similar in nature to the principal constituent of Cobra venom, acting by paralysing the respiratory centre. C. J. MARTIN (4), working in Australia, discovered the intravascular clotting of the blood produced by the *Pseudechis* (a Colubrine snake), and he suggested that the rapid death with convulsions produced by the Indian (RUSSELL'S) Viper is produced by such clotting in the blood vessels, while LAMB and HANNA (5), working in Bombay, have since found this to be the case, and have carefully studied the blood changes produced by the Indian Viper, coming to the conclusion that clotting of the blood in the case of rapid deaths, and loss of coagulability and hæmolytic effects in the slow ones, are the essential action of the poison, and that this Viperine venom has no primary action on the respiratory centre. D. D. CUNNINGHAM (11) had previously published careful observations on the effect of RUSSELL'S Viper on the blood, and attributed the convulsions to a direct action on the central nervous system.

Whilst working recently at the action of the poisons of the Hydrophidæ and Indian poisonous Colubrine snakes on the respiration and circulation, I made some tracings illustrating the action of RUSSELL'S Viper, which soon led me to think that there must be some important factor, other than the blood changes, which had previously escaped notice, namely, a rapid failure of the circulation in the absence of any intravascular clotting. Through the kindness of several friends I have been able to extend my inquiry, with important results, from *Daboia Russellii* to the African Puff Adder, the *Crotalus horridus*, or Rattlesnake, and to one of the Indian representatives of the last named, viz., *Trimeresurus anamallensis*.

I. *Daboia Russellii*.

This snake is very common in the thickly populated parts of Bengal and Assam, and after the Cobra probably causes more loss of life than any other in India, while we have as yet no efficient antivenin for use in the treatment of its bites. In man great extravasation of bloody serum takes place throughout the limb affected, together with hæmorrhage from the bowel, and death does not usually take place

until after several hours or even a day or two, when the blood within the body is fluid, as first pointed out by FAYRER (2), and no intravascular clotting is found, as, relatively to the small size of the animals usually killed by this snake, man is a very large one. In smaller animals even subcutaneous injections of the venom produce, after a time varying inversely with the dose, violent convulsions and rapid death, while very minute amounts given intravenously produce the same effects. In both cases the blood is found to be clotted within, especially the portal veins, the pulmonary arteries and the right side of the heart, as shown by LAMB (5). D. D. CUNNINGHAM (11), however, showed some years ago that by repeated injections of slightly sub-minimal lethal doses at intervals of 20 minutes or so, a slow form of poisoning without convulsions, but with hæmorrhages similar to those which occur in man, could be produced. After death in such a case there will be no intravascular clotting found. Again, WALL (3) pointed out years ago that if Daboia venom is heated for a short time to 100 F. its action appears to be altered, the primary convulsions no longer appearing, but a gradual failure of respiration occurs terminating in respiratory convulsions of a secondary nature, much as in many cases of poisoning by the venom of the Rattlesnake. The above references suffice to show what different opinions have been held as to the action of the Daboia venom, while the whole of the known facts appear to be difficult to satisfactorily explain on any hypothesis yet put forward. In order to try and throw some light on the question I have taken simultaneous tracings of the respirations and blood pressure curves with interesting and instructive results.

Blood Pressure and Respiratory Curves.

EXPERIMENT I.—In the first experiment a sufficient dose was given intravenously to produce rapid death with primary convulsions. A Cat, weighing 2 kg., was chloroformed and 2 mg. (1 mg. per kilogramme) was injected into the external jugular vein, the blood pressure of the carotid artery and the respirations, by means of a tracheal tube connected with a SANDSTRÖM'S recorder, being taken.

Time.	Blood pressure.	Respirations.		Remarks.
		Per $\frac{1}{2}$ minute.	Amplitude.	
	millims.		millims.	
Before injection	90	16	8	
After $\frac{1}{2}$ min.	50	24	9	
" 1 "	25	33	4	
" 1 $\frac{1}{2}$ "	10	7	5	Convulsions.
" 2 "	5	2	—	
" 3 "	5	2	—	Terminal respiratory gasps.

Post-mortem.—The portal vein and its tributaries were solid with blood clot. The blood in the venæ cavæ and their tributaries, as well as in the right side of the heart and the pulmonary arteries was also clotted, but the left side of the heart was free from it. The diaphragm contracted well when the phrenic nerves were stimulated with the secondary coil at a distance of 45 millims. and the sciatics responded at 23 millims. showing no paralysis of the motor end-plates, as occurs in poisoning by the Hydrophidæ and Colubrine snakes.

Here we have a typical example of rapid death from Daboia poisoning with a vascular clotting, and the tracing shows precisely the opposite effects to those produced by the Cobra class of venoms, namely, a primary failure of the circulation with temporary increased rapidity of the respirations, followed by secondary respiratory failure and terminal respiratory convulsions, whereas in Cobra poisoning the respiration fails first and the circulation only secondarily to the cessation of the breathing. The intravascular clotting found *post-mortem* would appear to be sufficient to account for the symptoms here seen.

EXPERIMENT II.—In this case a sufficient dose to kill fairly rapidly, when injected subcutaneously, was used. A Rabbit, weighing 1 kg., was chloroformed and injected subcutaneously in the muscles of the right thigh with 5 cg. of Daboia venom, dissolved in 1 cub. centim. of 0.9 per cent. NaCl solution, records being taken, as before, of the blood pressure and respirations.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . . .	85	80	
After 1 min.	85	81	
" 2 mins.	85	82	
" 3 "	80	81	
" 4 "	75	79	Blood pressure falling.
" 5 "	65	79	
" 6 "	55	78	
" 8 "	40	88	
" 10 "	40	80	
" 12 "	40	80	
" 16 "	50	78	
" 20 "	40	—	
" 24 "	35	70	Respiration slowing.
" 30 "	30	64	
" 32 "	20	60	
" 33 "	10	56	
" 34 "	0	23	
" 35 "	0	0	

Post-mortem.—Clots were found in both the portal and systemic veins, the right heart and pulmonary arteries and a little in the left auricle and thoracic aorta.

Only a few drops of blood could be obtained from the large veins, and it did not clot after 24 hours. The phrenic nerves were not paralysed.

Here, again, the circulation failed, but in a much more gradual manner than in the first case where the poison was injected intravenously. The respirations remained unaffected until after the blood pressure had become greatly decreased, when they became reduced in number minute by minute until they ceased. If no blood-pressure tracing had been taken and the vessels had not been searched for intravascular clotting after death, it might easily have been regarded as a case of primary respiratory failure, instead of one of primary failure of the circulation produced by intravascular clotting, followed by secondary affection of the respiration due to deficient supply of blood to the medullary centres.

EXPERIMENT III.—WALL's experiment of heating Daboia venom to 100° C. for a short time was repeated, in order to see how far the respiratory failure thus produced is primary or secondary. A Cat, weighing 2·8 kg., was chloroformed, and 2·8 cg. of Daboia venom (1 cg. per kilogramme), which had previously been heated for one minute to 100° C., was injected into the external jugular vein, and records taken as before.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . . .	150	41	Blood pressure falling.
After $\frac{1}{2}$ min.	125	—	
" 1 "	90	43	
" 2 mins.	75	43	
" 3 "	70	35	Respirations slowing.
" 4 "	65	35	
" 6 "	60	30	
" 10 "	55	21	
" 15 "	52	16	
" 20 "	55	8	
" 29 "	50	7	
" 30 "	25	3	
" 31 "	10	0	

Post-mortem.—There was much black clot in the portal and systemic veins, all the cavities of the heart, and in the pulmonary and aortic arteries. Blood from the carotid artery clotted only feebly and imperfectly, forming a soft clot after some hours. The phrenic nerves were not paralysed.

Here we have just the same gradual failure of respiration as WALL described under the same conditions, but with the addition of a blood-pressure tracing, which clearly shows that the circulatory failure was the primary affection, a fall to one-half having been registered within 2 minutes of the injection, while the respiratory failure did

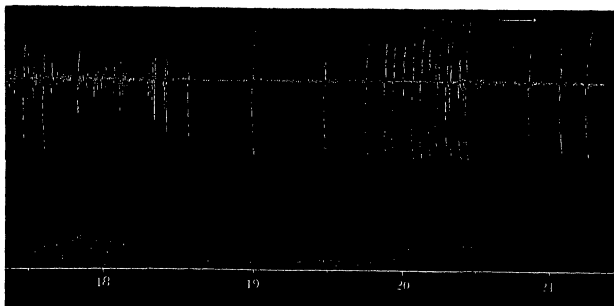
not begin until after that time, and was evidently secondary to the circulatory failure.

So far, then, the conditions met with in the three preceding experiments can be explained as the result of intravascular clotting, and they afford no evidence of any direct effect of the poison on the respiratory centre. We have seen, however, that in man this intravascular clotting has not been found, but a loss of coagulability and hæmorrhages constitute the essential symptoms observed, and the question arises whether such deaths can be fully accounted for by the effects of the poison on the blood, namely, the loss of coagulability and a less marked hæmolytic action, or whether there is any other factor present. In order to simulate this condition, CUNNINGHAM's method of giving repeated slightly sub-minimal lethal doses must be used, or small doses may be given subcutaneously, which are not sufficient to produce intravascular clotting, but will bring about the negative phase of diminished coagulability of the blood, and then a small lethal dose may be given intravenously. In the following experiments these methods have been used with most instructive results.

EXPERIMENT IV.—A Cat, weighing 2.25 kg., was given chloroform followed by ether, and 0.225 mg. of Daboia venom (0.1 mg. per kilogramme) was injected into the external jugular vein, and records taken as before.

Time.	Blood pressure.	Respirations per minute.	Remarks.
Before injection . . .	millims.	35	
After 1 min.	110	32	
" 3 mins.	110	33	
" 6 "	110	30	
" 7 "	80	23	Blood pressure falling.
" 8 "	40	7	Respirations stopped.
" 9 "	40	—	Respiratory convulsions.
" 10 "	125	—	Blood pressure rising.
" 11 "	40-130	16	Traube-Hering curves.
" 13 "	70-110	21	
" 14 "	30	14	Fall of blood pressure.
" 15 "	30	1	Respirations stopped again.
" 16 "	100	—	Respiratory convulsions.
" 18 "	25-110	15	Blood pressure rising.
" 19 "	30	—	Traube-Hering curves.
" 20 "	75	—	3rd fall of pressure.
" 22 "	50-130	—	Respiratory convulsions.
" 25-26 mins.	25	—	Traube-Hering curves.
" 27 mins.	80	—	4th fall of pressure.
" 28 "	50-100	—	Respiratory convulsions.
" 30-31 mins.	20	—	Traube-Hering curves.
" 33 mins.	80	—	5th fall of pressure.
" 35 "	85	—	Respiratory convulsions.
" 37 "	45	—	Rise of blood pressure.
" 38 "	10	—	Final fall of pressure.
			Death.

(See Tracing III. for 18-22 minutes and 34-38 minutes.)



Tracing III. (*Daboia Russellii*, Experiment IV.).—Cat. *Daboia* venom. 0.1 mg. per kilogramme, intravenously. 18–21 minutes.

Post-mortem.—A few minutes after the final fall of blood pressure and cessation of respiration the body was opened. A few drops of blood only were obtained from the carotid artery, which was very dark, but clotted well and gave out fairly clear serum. The large systemic veins were greatly distended with dark fluid blood, which clotted fairly well in test-tubes. The portal system was full of fluid blood, but there were no clots in it or in the systemic veins. The heart continued to beat, the right side being distended with blood, and the left empty. The pulmonary and aortic arteries were free from blood clot, but on opening up the branches of the pulmonary artery within the lung a tiny clot was found in one small branch only. The brain was normal, and there was no sign of hæmorrhage in the medulla or elsewhere. Stimulation of the phrenic nerves with the secondary coil at 45 millims. produced good contraction of the diaphragm.

The above experiment presents several points of interest. In the first place death clearly due to circulatory failure resulted from a very small dose of the venom given intravenously, without there being any marked intravascular clotting which could account for it. The first fall of blood pressure, however, did not occur until after 6 minutes, instead of within a few seconds as in the first experiment, when ten times as large a dose was given. Immediately after the fall in blood pressure the respirations rapidly failed, and after another minute, what appeared to be terminal respiratory convulsions, commenced. During this interval of very low blood pressure (40 millims.) the heart continued to beat slowly, each beat being clearly recorded, showing that the heart itself was not paralysed. After a few convulsive respirations had taken place the blood pressure began to rise again and the heart to beat more rapidly, while less deep, but irregular, respirations began once more, and at the same time very marked waves of alternately rising and falling of the blood pressure, resembling Traube-Hering curves, appeared.

A close comparison of the curves showed that the rises of pressure immediately

followed a deep respiration or group of respirations, while the falls corresponded with temporary cessation of the breaths, as if the pressure was pumped up with each convulsive respiratory effort. In the middle of the 18th minute the pressure sank again, this time to only 30 millims., and the respirations ceased, but at the end of the 20th minute another series of convulsive respirations was followed by a second rise of the blood pressure and another series of waves with a very definite relationship to the now infrequent inspirations. This sequence of events was repeated three times more, as the figures above given show, but the amplitude of the pressure waves became less each time, and at the end of the 37th minute the pressure finally fell, the succeeding convulsive inspirations failing to improve it, and death ensued. At each of the pressure failures the record of the pulse beat became less and less marked, and was no longer evident in the final fall.

The absence of the intravascular clotting (except very slight in the pulmonary vessels), *post-mortem* in this case was somewhat surprising, and made it very difficult to account for the facts observed on the theory that they were due to the blood changes known to be produced by Daboia venom, unless it might be that there was clotting in the capillary vessels of the lung producing obstruction of the circulation, which was temporarily overcome by the greatly increased intrathoracic pressure produced by the respiratory convulsions. This appeared to me the most probable explanation of the curve, and it became necessary to devise some means of testing its truth. In order to do this I determined to repeat the experiment and after death to see what pressure was required to pass fluids through the pulmonary circulation, so as to be able to form a rough estimate of the degree of obstruction, if any, present in them, which might possibly be due to capillary clotting.

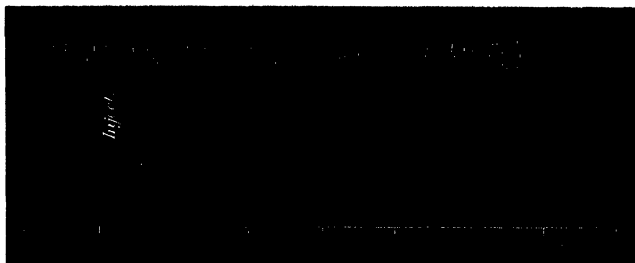
EXPERIMENT V.—A Cat, weighing $3\frac{1}{4}$ kg., was chloroformed and 0.325 mg. of Daboia venom (0.1 mg. per kilogramme) injected into the jugular vein.

Time.	Blood pressure.	Respirations per minute.
	millims.	
Before injection . . .	160	57
After 2 mins. . . .	140	58
" 6 "	135	54
" 11 "	150	44
" 21 "	180	36
" 33 "	165	32
" 44 "	170	38

At this point a second dose of 0.1 mg. per kilogramme was injected into the jugular vein with the following result :—

Time.	Blood pressure.	Respirations per minute.
After $\frac{1}{2}$ min.	millims. 160	—
" 1 "	145	32
" $1\frac{1}{2}$ mins.	140	—
" 2 "	115	38
" $2\frac{1}{2}$ "	75	—
" 3 "	40	31
" 4 "	30	0
" 7 "	20	—

(See Tracing IV. for 44–48 minutes.)

Tracing IV. (*Daboia Russellii*, Experiment V.).—Cat. Daboia venom. 0.1 mg. per kilogramme, intravenously, repeated after 44 minutes. 44–47 minutes.

Post-mortem.—On opening the chest the heart was beating, the right side being distended with blood and the left nearly empty. The systemic veins were also distended with fluid blood, which was very dark and did not clot in a test-tube. No clots could be found in the portal, pulmonary or systemic vessels, or in the heart. The diaphragm contracted well when the phrenic nerves were stimulated with the secondary coil at 45 millims. In order to test if there was any capillary clotting in the portal or pulmonary systems, normal salt solution was transfused through them from measured heights above the liver and lung respectively, with the result that it passed through readily at a pressure of 20 millims. of water in the case of the portal circulation, and of 30 millims. in that of the pulmonary vessels. It was therefore evident that there could be no obstruction of the capillaries of either organ such as could account for the circulatory failure recorded above.

In the foregoing experiment only a slight and temporary reduction of blood pressure followed the first very small intravenous dose, but on repeating it a rapid circulatory failure ensued, followed by the usual secondary failure of respiration. Yet there was absolutely no intravascular clotting, and the blood remained completely fluid after removal from the veins for over 24 hours.

Capillary clotting in the pulmonary or portal circulations was also excluded. The first dose evidently produced the negative phase of reduced coagulability of the blood, while the second one acting cumulatively brought about a rapid failure of the circulation. The accumulation of blood in the venous system, and the emptiness of the left heart and systemic arteries, were the most marked *post-mortem* features, while death had occurred far too rapidly to allow of the hæmorrhages, which are the characteristic feature of the chronic form of Daboia poisoning. It was now clear that there was some important factor present other than the mere changes in the coagulability of the blood, and further experiments were undertaken to see if, by first inducing the negative phase of reduced coagulability by repeated subcutaneous injections of sub-minimal lethal doses, rapid circulatory failure could be easily obtained by small intravenous doses without any intravascular clotting.

EXPERIMENT VI.—A Cat, weighing 8 kg., was given chloroform followed by ether, and 6 mg. of Daboia venom (2 mg. per kilogramme) was injected subcutaneously into the thigh.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	180	53	Respirations less frequent.
After 2 mins. . .	180	49	
" 12 " . .	195	38	
" 28 " . .	195	28	
" 30 " . .	—	—	
2nd subcutaneous injection of 2 mg. per kilogramme.			
After 36 " . .	175	28	
" 46 " . .	165	30	
3rd injection of 0.3 mg. (0.1 mg. per kilogramme) intravenously.			
After 1 min. . .	150	32	
" 3 mins. . .	160	34	
" 5 " . .	150	23	
4th injection of 0.6 mg. (0.2 mg. per kilogramme) intravenously.			
After 1 min. . .	110	28	Fall of blood pressure.
" 2 mins. . .	70	41	
" 3 " . .	60	34	
" 5 " . .	40	25	Respirations stopped. Terminal respiratory convulsions.
" 6 " . .	30	8	
" 7 " . .	30	3	

Post-mortem.—The portal, pulmonary and systemic circulations, together with the heart, were all found to be free from blood clot. There were local extravasations

of blood-stained serum at the seats of subcutaneous injection. The phrenic nerves were not paralysed.

Here, again, a rapid failure of the circulation was produced by a very small dose of the poison intravenously after subminimal lethal doses subcutaneously, while the respirations, although much reduced in frequency after the first doses, only failed subsequently to the final marked fall of blood pressure; yet there was no intravascular clotting to account for the result. In the next experiment, repeated and increasing doses of Daboia venom were given subcutaneously to see if a marked reduction in the blood pressure could be induced by this mode of administration, which is the natural one.

EXPERIMENT VII.—A Cat, weighing 3.5 kg., was given chloroform followed by ether, and 2 mg. per kilogramme of Daboia venom injected subcutaneously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	100	33	
After 10 mins. . .	115	24	
„ 20 „ . .	125	20	Respirations less frequent.
2nd subcutaneous injection of 5 mg. per kilogramme.			
After 40 „ . .	120	20	
3rd subcutaneous injection of 5 mg. per kilogramme.			
After 50 „ . .	120	24	
„ 69 „ . .	120	22	
4th subcutaneous injection of 10 mg. per kilogramme.			
After 79 „ . .	100	22	
„ 89 „ . .	90	20	Blood pressure falling.
„ 97 „ . .	85	20	
5th subcutaneous injection of 20 mg. per kilogramme.			
After 108 „ . .	65	20	Blood pressure falling.
„ 144 „ . .	65	22	
„ 152 „ . .	70	24	
6th injection of 0.1 mg. per kilogramme intravenously.			
After 1 min. . .	65	24	
„ 2 mins. . .	55	23	
„ 3 „ . .	30	37	
„ 4 „ . .	25	32	
„ 6 „ . .	25	26	
7th injection of 0.1 mg. per kilogramme intravenously.			
After 8 „ . .	25	21	
„ 9 „ . .	20	10	
„ 10 „ . .	20	0	Respiration stopped.

Post-mortem.—There was no intravascular clotting anywhere. The blood on removal formed only a very loose clot after some time, which exuded only slightly hæmoglobin-stained serum. The phrenic nerves were normal. On carrying out transfusion with normal salt solution it was found to pass readily through the pulmonary circulation at a pressure of 30–40 millims., through the portal system at one of 30 millims., and through the abdominal aorta and back by the inferior vena cava at one of 30 millims., all water pressure, proving that the failure of the circulation was not due to any intravascular clotting in the capillary vessels.

Here, again, we have death from primary circulatory failure without any clotting in the vessels to account for it. The first three small subcutaneous doses no doubt produce a condition of reduced coagulability which prevented the subsequent ones from causing intravascular clotting. The fourth and fifth larger subcutaneous ones produced a marked fall of pressure of a very gradual nature until it reached about half the normal, so that there is little reason for doubt that in time fatal circulatory failure without any clotting could be brought about in this way (as was done in a later experiment, namely No. XI.). In order to terminate the experiment more quickly, very small intravascular doses were given, when a similar rapid circulatory failure to those of the previous experiments resulted.

The above experiments were sufficient to show that if steps are taken to produce a reduced coagulability of the blood by small doses, then a fatal circulatory failure independent of any intravascular clotting can readily and constantly be induced, which will be of a rapid nature if the later dose be introduced into the circulation directly, and of a much slower character if they be given subcutaneously as in nature. It remained to ascertain the exact nature of the circulatory failure, and if it will account for the hæmorrhages which constitute the essential symptoms of the slow form of poisoning by Daboia venom which occurs in the case of man. At first sight the rapid fall of blood pressure following very small intravenous injections of the poison seemed to indicate a direct action on the heart, but against this interpretation was the remarkable recovery of the pressure with the onset of respiratory convulsions which repeatedly occurred in Experiment III. and to a less extent in some others, together with the evidence of the records of the continuance of the pulse beats during and sometimes for minutes after the fall of the blood pressure. Further, the marked Traube-Hering curves pointed to vaso-motor action, and a paralysis of the vaso-motor centre would equally well explain the blood-pressure fall.

Direct Action on the Heart.

The effect of direct applications of solutions of Daboia venom in normal salt solution to excised frogs' hearts was therefore tried, with the result that even solutions of a strength of 1 in 50 produced no definitely retarding effect on the contractions of the organ, which made it difficult to conceive how the injection of 0.1 mg. per

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kilogramme weight could cause an immediate great fall of blood pressure by the direct action of the venom on the heart. Attention was then turned to the question whether the effects recorded above could be explained as a result of a direct paralysing effect of the poison on the vaso-motor centre in the medulla, for which purpose the following experiments were performed.

Action on the Central Vaso-Motor Centre.

EXPERIMENT VIII.—A Dog, weighing 10 kg., was given chloroform followed by ether, the spinal cord cut in the cervical region, and artificial respiration performed. The blood pressure before the section was 135 millims. of mercury, and it fell afterwards to 65 millims. A first subcutaneous injection of 2 cg. of Daboia venom (2 mg. per kilogramme, a sub-minimal lethal dose) was now injected into the thigh, in order to produce a reduction in the coagulability of the blood and prevent a second intravenous dose from killing immediately by clotting the blood in the vessels, so as to allow of the effect of this second dose on the pulse and blood pressure being observed. The second dose consisted of 1 mg. per kilogramme (1 cg.) in 0·2 cub. centim. salt solution, and was injected into the external jugular vein. The following table shows the result of the intravenous injection :—

Time.	Blood pressure.	Pulse rate per minute.	Respiration.
	millims.		
Before injection . . .	50	72	Artificial.
After 1 min.	50	68	Artificial.
" 2 mins.	45	49	Artificial.
" 3 "	45	47	Artificial.
" 4 "	40	—	Artificial.
" 5 "	30	—	Artificial.
" 6 "	20	—	Artificial.
" 7 "	10	—	Artificial.

Post-mortem.—The heart was still beating feebly. There was extensive clotting in the portal veins, pulmonary arteries and systemic veins, as well as some in the right heart. Here the slow steady failure of the circulation was doubtless due to intravascular clotting, which would have taken place in a few seconds with the dose used, but for the first subcutaneous injection of venom. The absence of any sudden fall of blood pressure and the continuation of the pulse after this large intravenous dose shows that the venom does not produce the falls always caused by it when the cord is intact by direct action on the heart, and the fact that the production of vaso-dilatation by section of the cord prevents any sudden marked fall of pressure due to the intravenous injection of the venom indicates that its action is one of paralysing the central vaso-motor centre in the medulla, and not a direct one on the heart.

If this is the case, then section of the cord after a very marked fall of pressure produced by Daboia poison should not cause any further very marked fall of pressure. Further, if central vaso-motor paralysis is the cause of the pressure fall, then it ought to be possible to observe the dilatation of the blood vessels of the portal area in the mesentery and omentum. In order to test these points the following experiment was performed.

EXPERIMENT IX.—A cat, weighing 3 kg., was given chloroform followed by ether. Two subminimal lethal subcutaneous doses of Daboia venom were injected, in order to produce the negative phase of reduced coagulability of the blood before giving an intravenous dose. They were as follows: First injection, 6 mg. in 0.5 cub. centim. salt solution (2 mg. per kilogramme), and after 15 minutes a second injection of 12 mg. (4 mg. per kilogramme). No definite effect on either the blood pressure or respiratory rate was produced by these small doses. At 42½ minutes after the first dose, 0.3 mg. (0.1 mg. per kilogramme) was injected into the external jugular vein with the following result. Just before this last dose a piece of the omentum was withdrawn from the abdominal cavity, and, protected by cotton wool soaked in warm normal saline solution, was placed under the microscope and the circulation watched during and after the fall in blood pressure produced by the intravenous injection. The field under observation included a small artery and vein, from which two or three small vessels containing circulating blood came off. With the rapid fall in pressure, which occurred within 2 minutes of the third injection, a very marked dilatation of both the large and the small vessels was noted, while numerous capillaries, which had previously been invisible, were now seen with blood streams actively flowing through them. The circulation continued actively until after the cord was cut 11 minutes after the third injection. The effect on the circulation is shown in the following figures:—

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before 3rd injection . .	150	48	Rapid fall of blood pressure.
After ½ min.	140	—	
" 1 "	70	56	
" 2 mins.	50	56	
" 8 "	50	45	
" 11 "	50	37	
* Spinal cord cut in the cervical region. Artificial respiration.			
" 12 "	30	—	Artificial respiration left off.
" 15 "	30	—	
" 17 "	40	—	
" 18 "	50	—	
" 20 "	40	—	
" 22 "	10	—	

Post-mortem.—The large veins were distended with incoagulable blood. There was a blood clot in the external jugular vein just below the seat of injection only, while a very small clot was found in one of the tributaries of the superior mesenteric vein. The portal vein and its distributory vessels within the liver were quite free from clots, as was the pulmonary circulation and the cavities of the heart. The small clots noted must almost certainly have been formed as a result of the preliminary subcutaneous injections, and even if they were formed after the third intravenous one they would in no way account for the rapid fall of blood pressure from 150–50 millims. within 2 minutes of the last injection. On section of the cervical cord there was a slight further fall of pressure, which was completely recovered from after artificial respiration had been kept up for a few minutes, so it must have been solely due to shock. It is evident, therefore, that complete vaso-motor paralysis must have resulted from the action of the intravenous injection of the Daboia venom, for the heart continued to beat steadily until several minutes after the artificial respiration was finally stopped. The observation of the very marked dilatation of the vessels of the omentum confirms the view that the fall of pressure was due to a vaso-motor dilatation of very marked degree. Immediately after death the blood was transfused through the portal and pulmonary circulations, and was found to pass easily from a height of 40 millims., showing that there was neither capillary clotting or any change in the viscosity of the blood which could account for the failure of the circulation.

Simultaneous General Blood-Pressure Fall and Portal Vaso-Dilatation.

In order to obtain an absolute demonstration of the vaso-dilatation of the portal vessels, simultaneous tracings of the general blood pressure and of the variations in the amount of blood in a large loop of the small intestine placed in an oncometer connected up with a Sandström recorder were taken in cases of poisoning with different Viperine venoms, Dr. T. G. BRODIE very kindly helping me with the first (that with the Puff Adder venom),

EXPERIMENT X.—A Cat, weighing 4 kg., was chloroformed and a cannula in the carotid artery was connected up with a GAD's manometer. The abdomen was then opened and a large loop of intestine was isolated by ligaturing in two places on either side, and placed in an oncometer connected up with a SANDSTRÖM's recorder. Any vaso-dilatation would cause the lever to rise, and any cause of diminished blood entry would cause it to fall. The following is the record of the experiment:—

Time.	Blood pressure.	Oncometer lever.	Remarks.
Before 1st injection	millims. 160		
1st injection of 0·1 mg. per kilogramme intravenously in 0·5 cub. centim. normal saline.			
After $\frac{1}{2}$ min. . . .	150	Rising very fast.	Vaso-dilatation.
" 1 "	125	Rising very fast.	Vaso-dilatation.
" 2 mins. . . .	87	Sudden fall.	Clotting in arterial cannula. Fall due to clotting in mesenteric vein.
" 3 "	85	Rising fast.	Vaso-dilatation.
" 6 "	85	Rising steadily.	Vaso-dilatation.
" 9 "	87	Rising steadily.	Vaso-dilatation.
2nd injection of 0·2 mg. per kilogramme intravenously in 0·5 cub. centim. normal saline.			
After 10 mins. . . .	87	Rising slowly.	
" 11 "	75	Slight fall.	
" 12 "	70	Rising slowly.	
" 15 "	87	Rising slowly.	
" 18 "	90	Stationary.	
" 23 "	80-125	Rising steadily.	Traube-Hering curves well marked.
" 25 "	90-100	Rising steadily.	Traube-Hering curves less marked.
" 28 "	70	Rising slowly.	
" 30 "	65	Stationary.	

(See Tracing V. for 1-3½ minutes.)

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Tracing V. (*Daboia Russellii*, Experiment X.).—Cat. Daboia venom. 0·1 mg. per kilogramme, intravenously. First 4½ minutes.

The pulsation of the vessels in the loop of intestine became very feeble by this time, so the record was stopped.

Post-mortem, slight clotting was found in the external jugular vein below the seat of injection. The portal vein and its branches in the liver were free from clot, as was the pulmonary circulation. Careful search showed a small thread of clot in one of the tributaries of the mesenteric vein within the oncometer. This accounts for the sudden fall of pressure in the oncometer at the end of the 2nd minute, at the same time that clotting occurred in the carotid artery cannula. Except for this, marked vaso-dilatation of the portal vessels occurred throughout the 10 minutes following the first injection of the Daboia venom, and to a somewhat less extent after the second one, the entry of which was probably retarded by the clot in the external jugular vein just below the cannula through which the injections were made. The secondary marked vaso-dilatation, which occurred with the onset of the Traube-Hering curves at the 23rd minute, is also of great interest, as these curves always make their appearance before the final fall of blood pressure, and are doubtless due to the struggles of the enfeebled vaso-motor centre to keep up its tonic contraction of the peripheral vessels.

This experiment, then, proves that marked vaso-dilatation of the portal system does occur coincidently with the general fall of blood pressure for which it accounts. In view, however, of the effect of Viperine poisons in causing hæmorrhages, which in the case of the Rattlesnake in particular has been shown by WEIR MITCHELL and REICHERT (9), and more recently by FLEXNER (10), to be largely due to a destructive effect of the poison on the endothelial lining of the blood vessels, it becomes of importance to determine if the vaso-dilatation produced by the Daboia venom is due in part at least to an action on the peripheral vessels and not entirely to a paralysing action on the central vaso-motor centre in the medulla.

The Effect of Daboia and Cobra Venoms on the Calibre of the Systemic Vessel.

Dr. J. G. BRODIE very kindly tested the action of these venoms on the peripheral mechanism, controlling the circulation through the blood vessels by means of his perfusion apparatus.

EXPERIMENT I.—The hind quarters of a freshly killed cat were perfused with defibrinated blood of cats through the abdominal aorta. Ten mg. of Cobra venom in 1 cub. centim. of 0·9 per cent. salt solution was injected into the circulating blood. A rapid and very marked vaso-constriction immediately occurred, and persisted for so long that no further experiments could be carried out with this animal.

EXPERIMENT II.—The small intestines of another cat were perfused with blood, and 0·3 mg. (0·1 mg. per kilogramme in 1 cub. centim. salt solution) were injected into the circulating blood. A very rapid and marked vaso-constriction appeared before the whole of the injection had been made. After some time a second injection of 3 mg.

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in 1 cub. centim. salt solution was injected, and, although the vessels were still contracted to a considerable degree, a further marked constriction occurred, the amount of blood returning through the cannula in the inferior vena cava becoming visibly less.

Thus we have a large dose of Cobra venom and a small one of Daboia venom both causing a marked local vaso-constriction when perfused through blood vessels which were cut off from the influence of the vaso-motor centre. This effect is worthy of further study, but at least it is clear that the vaso-dilatation produced by the injection of Daboia venom is not due to any action on the peripheral mechanism, but solely to paralysis of the central control of the medulla, acting in spite of the opposite tendency to constriction due to the peripheral action of the venom. This conclusion is also supported by the action of drugs which produce local constriction of the blood vessels, of which the most powerful is adrenal extract. The following experiment will serve to illustrate this point :—

The Effect of Abdominal Pressure and Adrenal Extract on the Blood Pressure in Daboia Poisoning.

EXPERIMENT XI.—Cat, weighing $2\frac{1}{4}$ kg., was chloroformed and Daboia venom injected subcutaneously, small doses being first given to produce the negative phase of reduced coagulability. The first dose was 2 mg. per kilogramme.

Time.	Blood pressure.	Respirations per $\frac{1}{4}$ minute.	Remarks.
	millims.		
Before injection . .	150	39	
After 10 mins. . .	140	32	
2nd injection of 5 mg. per kilogramme subcutaneously.			
After 20 " " "	150	32	Respiration less frequent.
" 40 " " "	150	22	
" 73 " " "	125	21	
3rd injection of 20 mg. per kilogramme subcutaneously.			
After 5 " " "	100	20	Respiration stopped. Infrequent convulsive respirations. Infrequent convulsive respiration. Abdominal binder applied. Abdominal binder applied. Binder relaxed. Binder tightened.
" 7 " " "	90	—	
" 8 " " "	65	—	
" 10 " " "	40	17	
" 13 " " "	35	—	
" 20 " " "	40	—	
" 35 " " "	50	—	
" 37 " " "	55	—	
" 45 " " "	60	—	
" 50 " " "	50	—	
" 55 " " "	60	—	
Adrenal chloride (1 in 1000), 0.3 cub. centim. in 1 cub. centim. salt solution injected intravenously.			
After 62 mins. . .	80	—	
" 65 " " "	80	—	
" 70 " " "	75	—	
" 75 " " "	25	—	

This experiment is interesting from several points of view. In the first place we have a circulatory death produced entirely by subcutaneous doses of Daboia venom, the fall of pressure following the last large dose having been a rapid one of marked degree. *Post-mortem*, the blood was incoagulable as usual, the large veins being distended with it. There was some clotting in the portal system, together with fluid blood. The pulmonary circulation and heart were free from clot, as were the large systemic veins, with the exception of those near the seats of injection in the thighs and axilla, where there were also effusions of blood-stained fluid and petechiæ. The partial clotting in the portal circulation probably took place as a result of the first two injections, and would not account in any way for the failure of the general circulation. Secondly, the improvement of the blood pressure each time that pressure was applied to the abdomen by means of a binder was noteworthy, being no doubt due to the displacement of blood from the dilated portal vessels, and its being driven on to the heart. Lastly, the marked, although very temporary, improvement of the blood pressure immediately following the injection of the adrenal chloride, shows that the small arteries could still contract and raise the pressure even when it had reached a very low ebb.

We shall see later, when discussing the other Viperine poisons, that a very much more marked and prolonged effect on the pressure can be obtained by the use of this drug before the vaso-motor centre is completely paralysed, but the result above recorded supports the conclusion derived from the perfusion experiment that the peripheral vaso-motor mechanism is not destroyed by the Daboia venom, and that the fall in pressure noted must be due to paralysis of the central vaso-motor centre in the medulla. In the case of this venom, the difficulties due to the complications produced by the intravascular clotting it also produces rendered the demonstration of the paralysis of the vaso-motor centre a matter of considerable difficulty; but we shall see that with some of the other Vipers little or no intravascular clotting is produced, so that in them the circulatory failure can be much more easily studied. It will be well, then, to record some experiments with other Viperine poisons before summing up the effects of the Daboia venom, so as to be able to consider the physiological action of the Vipers as a whole.

II. *The African Puff Adder.*

Through the great kindness of Dr. J. W. W. STEPHENS, in placing at my disposal some venom of the Puff Adder, which he collected in Africa, while on the Malarial Commission of the Royal Society, and in allowing me to publish the records, I have been able to take some tracings illustrating the action of this venom for comparison with that of *Daboia Russellii*, with results of considerable interest. As in all the other experiments, the dried venom was used, and it has proved almost as deadly as Daboia venom itself when injected subcutaneously into pigeons, the symptoms being

also similar in the case of the two poisons, a lethal dose causing death with violent convulsions. *Post-mortem*, intravascular clotting was found most marked in the portal system, but also present in the pulmonary arteries, and in the systemic veins and heart, thus resembling again the changes found after acute Daboia poisoning. A dose of 5 mg. per kilogramme produced death in this way in 1 hour, and 4 mg. per kilogramme in 5 hours. The same result was obtained with 3 mg. of the Daboia-venom with which I have worked. With a single lethal dose, then, there is no marked difference between the action of the two venoms, while in both there is much local extravasation of bloody serum, which is somewhat more extensive in the case of the Puff Adder.

A difference between the two is, however, noticed as soon as repeated sub-minimal lethal doses are given, in order to produce the chronic form of poisoning which the bites of vipers cause in man. As already stated, this chronic form of poisoning was produced in fowls by D. D. CUNNINGHAM by repeated small doses of Daboia venom, the negative phase of reduced or lost coagulability of the blood being caused, and death eventually resulting from repeated hæmorrhages from the bowel, &c. This chronic condition I have not found easy to produce in the case of pigeons, probably on account of their small size as compared with fowls, intravascular clotting being often produced by repeated injections of sub-minimal doses, although it may be limited to the portal circulation, and not sufficient to cause death by itself. In the case of the Puff Adder poison, on the other hand, it is quite easy to produce fatal results by repeated slightly sub-minimal lethal doses without the production of any intravascular clotting, but with a complete loss of coagulability of the blood, and accompanied by very marked pericardial, endocardial, sub-pleural, mesenteric, and other hæmorrhages as marked in degree as those produced by Rattlesnake venom. In these cases very marked portal congestion is present, pointing to a vaso-motor paralysis such as we have seen is the essential result of the action of the Daboia venom if intravascular clotting is avoided by any means.

Blood Pressure and Respiratory Curves.

As in the case of the Daboia venom, tracings have been taken of the respirations and of the carotid pressure, and the following will illustrate the effect of a single lethal dose given intravenously.

EXPERIMENT I.—A Cat, weighing $2\frac{1}{2}$ kg., was chloroformed and a tracheal and a carotid cannula, respectively connected with recording apparatus, as in the former series of experiments. 5 mg. per kilogramme of Puff Adder poison were then injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection. .	140	41	Respirations stopped.
After $\frac{1}{2}$ min. . . .	125	—	
" $\frac{3}{4}$ "	115	21	
" $\frac{5}{8}$ "	60	—	
" 1 "	100	21	
" 2 mins. . . .	50	—	

Post-mortem.—There was clotting throughout the portal system, but it was not so complete as after one-fifth of the dose of Daboia venom, some fluid incoagulable blood being also present. The pulmonary arteries, right cavities of the heart and systemic veins also contained clotted blood together with a good deal of fluid blood. The phrenic nerves responded to a weak faradic stimulus. No hæmorrhages were found. Here we have intravascular clotting of a similar nature, although less marked in degree to that produced by the intravascular injection of one-fifth as much Daboia venom, although we have seen that there is but little difference in the minimal lethal dose of the two venoms when given subcutaneously. In the next experiment a small dose was first given, in order to produce the negative phase of reduced coagulability before injecting a larger dose.

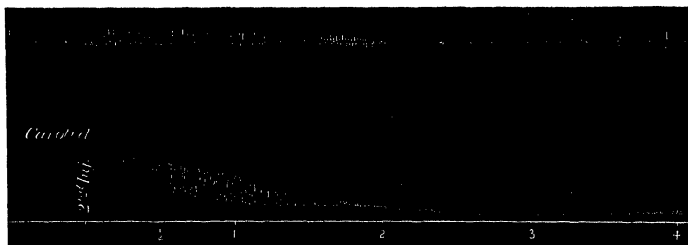
EXPERIMENT II.—A Cat, weighing 2·7 kg., was chloroformed and 0·3 mg. per kilogramme of Puff Adder poison injected intravenously, the usual records being taken.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . . .	145	33	Slight fall of blood pressure.
After 1 min. . . .	140	31	
" 5 mins. . . .	140	35	
" 10 "	125	30	
" 24 "	125	27	
2nd injection of 2 mg. per kilogramme intravenously.			
After $\frac{1}{2}$ min. . . .	95	—	Marked fall of blood pressure.
" 1 "	70	29	Respirations very shallow. Respirations stopped. Infrequent convulsive respirations. Infrequent convulsive respirations. Infrequent convulsive respirations. Infrequent convulsive respirations.
" $1\frac{1}{2}$ mins. . . .	55	—	
" 2 "	50	51	
" 3 "	40	0	
" 5 "	50	2	
" 12 "	50	4	
" 16 "	53	15	

EXPERIMENT II.—*continued.*

Time.	Blood pressure.	Respirations per minute.	Remarks.
Adrenal chloride (1 in 1000) 0.5 cub. centim. in 1 cub. centim. injected intravenously.			
After 17 mins. . .	millims. 60-125	4	Infrequent convulsive respirations.
" 20 " . .	55-100	3	Infrequent convulsive respirations.
" 23 " . .	55- 90	5	Infrequent convulsive respirations.
" 27 " . .	55	5	Infrequent convulsive respirations.
" 37 " . .	35	—	Infrequent convulsive respirations.
" 47 " . .	15	—	Infrequent convulsive respirations.
" 57 " . .	15	—	Respiratory convulsions ceased.

(See Tracing VI. for first 3 minutes.)



Tracing VI. (Puff Adder, Experiment II).—Cat. Puff Adder venom. 2 mg. per kilogramme, intravenously. First 4 minutes.

Post-mortem.—The large veins were distended with incoagulable blood. No trace of clotting could be found in the portal, pulmonary or systemic circulations or in the heart. The pericardium contained blood-stained fluid, and there were very marked sub-pericardial hæmorrhages over the outer surface of the heart, as well as beneath the endocardium, especially of the left ventricle. The mesentery, omentum and other parts of the peritoneum showed very numerous petechial hæmorrhages, such as commonly result from intravenous injections of Rattlesnake venom, but which I have never seen in the case of Daboia poisoning, except slight petechiæ in the left ventricle after prolonged poisoning.

In the above experiment we have the same circulatory failure without any intravascular clotting that has been obtained with Daboia venom injected after the establishment of the negative phase of reduced congulability of the blood, only it is much more easy to produce this state in the case of the Puff Adder poison. Further, after complete vaso-motor paralysis has occurred a marked and somewhat prolonged raising of the blood pressure was obtained by the injection of adrenal chloride. The

increased excursus of the lever during the fall of blood pressure is typical of that due to vaso-motor failure as opposed to cardiac failure, this venom having been found to have a slightly stimulating effect on excised frogs' heart having been obtained with strong solutions of Puff Adder venom (1 in 100). In another similar experiment the fall of pressure was more gradual, as will be seen from the following data.

EXPERIMENT III.—A Cat, weighing $3\frac{1}{2}$ kg., was chloroformed and 0.3 mg. per kilogramme of Puff Adder venom injected intravenously, the usual records being taken.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	130	—	
After 7 mins. . .	125	24	
2nd injection of 3 mg. per kilogramme intravenously.			
After 1 min. . .	100	32	Fall of pressure commencing.
" 2 mins. . .	70-110	34	Greatly increased excursus of the pulse.
" 3 " . .	60-100	37	
" 4 " . .	55-85	40	
" 5 " . .	65	40	
" 7 " . .	55	33	
" 11 " . .	55	37	
Adrenal chloride (1 in 1000) 0.2 cub. centim. in 1 cub. centim. salt solution injected intravenously.			
After 12½ mins. .	95	42	Marked rise in pressure.
" 12 " . .	80	42	
" 13 " . .	60	40	
" 20 " . .	50	23	Bled to death from carotid.

Post-mortem.—The portal, pulmonary and systemic circulations and the heart were free from any clotting. The blood was incoagulable and bright red in colour. There were well marked sub-pericardial, endocardial, sub-pleural and peritoneal hæmorrhages.

Here we have a steady fall of pressure with greatly increased excursus of the pulse, the heart continuing to beat steadily throughout, and was still contracting well at the end of the experiment. The adrenal chloride caused a marked but temporary rise of pressure. The usual slow failure of respiration, secondary to the failure of the blood pressure was showing itself when the experiment was stopped. A central vaso-motor paralysis would account for all the facts observed, while the following experiment affords further strong support to this view of the action of the poison.

EXPERIMENT IV.—A Cat, weighing 2 kg., was chloroformed and 0.2 mg. of Puff Adder venom injected intravenously, the usual records being taken.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	140	—	
After 1 min. . .	130	—	
„ 10 mins. . .	135	57	
2nd injection of 2 mg. per kilogramme intravenously.			
After 1 min. . .	120	54	
„ 2 mins. . .	100	60	
„ 3 „ . .	70	60	
„ 4 „ . .	60	53	
„ 6 „ . .	52	57	
„ 8 „ . .	50	46	
„ 12 „ . .	50	45	Peripheral end of sciatic nerve stimulated by strong faradic current. No effect on blood pressure.
„ 13 „ . .	50	45	Central end of sciatic stimulated. No effect on blood pressure.
„ 18 „ . .	50	28	
Adrenal chloride (1 in 1000) 0.3 cub. centim. in 1 cub. centim. salt solution injected intravenously.			
After 19 mins. . .	125	35	Marked rise of blood pressure.
„ 20 „ . .	120	31	
„ 22 „ . .	95	31	Pressure falling again.
„ 24 „ . .	65	30	
„ 30 „ . .	50	33	
„ 40 „ . .	50	28	
Nicotine 1 mg. in 1 cub. centim. salt solution injected intravenously.			
After 41 mins. . .	110	—	Marked rise of pressure. Respirations very rapid.
„ 42 „ . .	75	56	
„ 43 „ . .	55	54	
„ 50 „ . .	45	34	
„ 60 „ . .	45	21	
Caffiene citrate 1 grain in 1 cub. centim. salt solution injected intravenously.			
After 61 mins. . .	55	31	
„ 63 „ . .	45	33	
„ 67 „ . .	35	29	
3rd injection of 2 mg. per kilogramme intravenously of Puff Adder venom.			
After 69 mins. . .	35	32	
„ 71 „ . .	35	26	
„ 72 „ . .	35	29	Respirations very shallow.
„ 73 „ . .	35	0	Respirations ceased.
„ 76 „ . .	55	—	Respiratory convulsions.
„ 80 „ . .	45	—	
„ 90 „ . .	25	—	Convulsive respirations ceased.

Post-mortem.—The large veins were distended with incoagulable blood. The

were no clots in the portal, pulmonary or systemic circulations or in the cavities of the heart. The peri- and endo-cardium, mesentery and omentum showed numerous petechial hæmorrhages. The brain and medulla were examined, but no naked eye changes were found. The phrenic nerves when stimulated with a weak faradic current produced good contraction of the diaphragm.

Here, again, we have a steady fall in blood pressure as the essential action of the venom. When this had fallen to about 50 mg. of mercury, stimulation of the central end of the sciatic nerve produced no rise of blood pressure, proving that the central vaso-motor centre was by this time completely paralysed. Yet, after this, adrenal extract produced a very marked rise of blood pressure, showing that the peripheral vaso-motor system was intact. Nicotine (the trial of which was suggested to me by SIR LAUDER BRUNTON) also produced a marked but temporary rise of pressure, and at the same time a marked stimulation of respiration was noted. Caffeine citrate had much less effect. The long persistence of the respirations, although of a feeble nature, after complete paralysis of the vaso-motor centre is also noteworthy, as was its rapid failure after the injection of the third dose of venom, for this points to the venom having some direct action on the respiratory centre in addition to its marked one on the vaso-motor one. The last three experiments, then, show that in the case of the Puff Adder poison the tendency to the production of intravascular clotting is a much less marked feature than it is with Daboia venom, as a small preliminary dose intravenously rapidly produces the negative phase of lost coagulability, and allows of uncomplicated vaso-motor paralysis being readily produced by a second larger dose. It only remained to demonstrate the dilatation of the portal system coincidently with the fall in blood pressure, for which purpose the following experiment was carried out:—

EXPERIMENT V.—A Cat, weighing 3 kg., was chloroformed, and after a cannula in the carotid artery had been connected up with a GAD's manometer, the abdomen was opened and a loop of small intestine, isolated and placed in an oncometer connected up with a SANDSTRÖM's recorder, so that any increase in the blood entry due to vaso-motor dilatation would cause the lever to rise and *vice versa*. A preliminary dose of 0.3 mg. per kilogramme was injected intravenously with the following results:—

Time.	Blood pressure.	Oncometer lever.	Remarks.
	millims.		
Before injection . .	150	Level.	
After 1 min. . . .	150	Rising slowly.	Slight vaso-dilatation.
" 2 mins. . . .	140	Rising slowly.	Slight vaso-dilatation.
" 4 "	140	Level.	
" 5 "	150	Level.	

EXPERIMENT V—*continued.*

Time.	Blood pressure.	Oncometer lever.	Remarks.
	millims.		
2nd injection of 5 mg. per kilogramme intravenously.			
After $\frac{1}{2}$ min. . .	125	Rising fast.	Marked vaso-dilatation.
" 1 " . . .	120	Rising fast.	Marked vaso-dilatation.
" 2 mins. . .	115	Rising fast.	Marked vaso-dilatation.
" 3 " . . .	90	Rising fast.	Marked vaso-dilatation.
" 4 " . . .	85	Rising slowly.	Slight vaso-dilatation.
" 7 " . . .	60	Rising slowly.	Slight vaso-dilatation.
Adrenal chloride (1 in 1000) 0.3 cub. centim. in 1 cub. centim. salt solution injected intravenously.			
After 8 mins. . .	130	Rising fast.	Passive dilatation.
" 12 " . . .	70	Falling slowly.	Passive contraction.

(See Tracing VII. for first 2 minutes.)

1000
0.3

0.3

2nd inj.
5 mg. per kg.

Tracing VII. (Puff Adder, Experiment V.).—Cat. Puff Adder venom. 5 mg. per kilogramme, intravenously.

By this time the pulsation of the oncometer lever had become very slight, and the experiment was terminated shortly after.

Post-mortem.—The blood was incoagulable, and no trace of clotting could be found in the mesenteric vessels, portal, pulmonary, and systemic circulations or in the cavities of the heart. Marked petechial hæmorrhages were found in the

pericardium, endocardium, especially of the left ventricle, and in the peritoneum, especially in the mesentery.

Here once more we have actual demonstration of a marked vaso-dilatation of the portal vessels occurring at the same times that the fall in the general blood pressure occurred and in proportion to its degree, both being slight after the first small injection, and both equally marked immediately after the second larger dose. Thus we have evidence of the same effect in paralysing the central vaso-motor centre by both Daboia and Puff Adder venoms, only, owing to the much less action of the latter in producing intravascular clotting of the blood, it is easier to demonstrate in the case of the Puff Adder.

B. CROTALIDÆ.

The Crotalidæ or Pit Vipers.

The best known poisonous snakes of this class are the American Rattlesnakes, while in India they are represented by the different species of *Trimeresurus*. I am indebted to Dr. J. BRUNTON BLAIR for some venom of *Crotalus horridus*, and to Dr. W. DOWSON for that of *Trimeresurus anamallensis*, which will serve very well for comparison with the two true vipers already dealt with.

I. *Crotalus horridus*.

A few experiments on pigeons showed that the respirations were first increased in number, and the bird appeared watchful and anxious as in the early stages of Daboia poisoning. Then the respirations gradually slowed down, but there was none of the sleepiness and nodding of the head, which is so characteristic of Colubrine poisoning. Lastly, convulsions occurred, and death quickly ensued. Except that the convulsions were usually less violent than in the case of Daboia poisoning, there was little difference between the general symptoms observed. *Post-mortem*, even after death had been produced in less than an hour by the subcutaneous injection of over ten times a fatal dose, no clotting in the portal or other vessels could be found, but the blood was incoagulable. A dose of 5 mg. per kilogramme caused death in about 10 hours.

Circulatory and Respiratory Curves.

Circulatory and respiratory tracings were taken as in the former experiments with the following results :—

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EXPERIMENT I.—A Cat, weighing $2\frac{1}{2}$ kg., was chloroformed and 1 mg. per kilogramme Rattlesnake venom injected intravenously, the usual records being taken. This dose in the case of Daboia venom produced very rapid death, with intravascular clotting; so it was first tried, as there is not much difference in the minimal lethal subcutaneous dose in pigeons of the two venoms.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	145	35	
After 1 min. . .	105	36	Marked fall in blood pressure.
" 2 mins. . .	95	27	
" 3 " . .	85	20	Respirations less frequent.
" 4 " . .	75	24	
" 5 " . .	65	23	
" 15 " . .	60	21	Traube-Hering curves well marked.
" 24 " . .	65	15	Traube-Hering curves well marked.
" 36 " . .	60	28	Traube-Hering curves less marked.

At this point the experiment was stopped and the animal bled to death from the carotid artery. *Post-mortem*, stimulation of the phrenic nerves with a weak induction shock (secondary coil at 45 millims.) caused good contraction of the diaphragm. The blood was bright red and incoagulable. No trace of clotting could be found in the portal, pulmonary or systemic circulations or in the heart. There was some blood-stained serum exuding from the wound.

Here we have the same fall of blood pressure, following a single injection of Rattlesnake poison, without a trace of intravascular clotting, which was also obtained in the case of the true Vipers by the injection of a large dose after reduced coagulability of the blood had been produced by preliminary small doses. The reduced frequency of the respirations followed the reduction in blood pressure, being probably secondary to it. At the time the animal was killed there was some tendency to improvement in both the blood pressure and the respiratory rate, and recovery might have taken place.

EXPERIMENT II.—A Rabbit, weighing $1\frac{1}{2}$ kg., was chloroformed, and 1 cg. per kilogramme of Rattlesnake venom injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	90	116	
After $\frac{1}{2}$ min. . . .	50	—	Marked fall of blood pressure.
" 1 "	60	113	
" 2 mins. . . .	50	123	Respirations increased.
" 4 "	40	116	
" 6 "	40	97	
" 10 "	35	80	Respirations slowing.
" 14 "	25	64	
" 26 "	25	54	
" 36 "	30	38	
" 40 "	30	13	
" 42 "	25	7	
" 46 "	15	3	Respirations ceased.

Post-mortem.—The blood was incoagulable. No clotting could be found in the portal, pulmonary or systemic circulation. The phrenic nerves were not paralysed. In order to see if there was any capillary obstruction, which could account for the failure of the circulation, the blood was transfused through both the pulmonary system and through the abdominal aorta, and was found to run through both readily from a height of 40 millims., proving that there was no capillary clotting present.

Here, again, we have a primary circulatory failure followed by slowing down and eventual failure of the respirations, due to the deficiency of blood circulating through the medulla, which seems to be sufficient to account for the respiratory failure without any direct effect of the poison on the breathing centre itself.

EXPERIMENT III.—A Rabbit, weighing 1·7 kg., was chloroformed and injected intravenously with 5 cg. per kilogramme.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	95	116	
After $\frac{1}{2}$ min. . . .	40	—	Marked fall in blood pressure.
" 1 "	35	145	Respiration quickened.
" 2 mins. . . .	25	115	
" 3 "	20	94	Respiration slowed and shallow.
" 4 "	15	84	
" 6 "	12	76	
" 12 "	10	42	
" 14 "	10	29	
" 15 "	0	4	Respiration ceased.

Post-mortem.—The heart was still beating feebly. The phrenics were not paralysed.

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The blood was dark and incoagulable. A clot was found in the external jugular vein just below the seat of injection only, and not extending its whole length. No other clots could be found in the portal, pulmonary or systemic circulations or heart. The fluid blood was easily transfused through the lungs and through the abdominal aorta from a height of from 30-40 millims. Here, once more, we have a primary circulatory followed by a secondary respiratory failure, both being more marked and rapid than with the smaller doses used in the two preceding experiments.

EXPERIMENT IV.—A Cat, weighing $2\frac{1}{2}$ kg., was chloroformed and injected intravenously with 5 cg. per kilogramme of Rattlesnake venom in 1 cub. centim. salt solution.

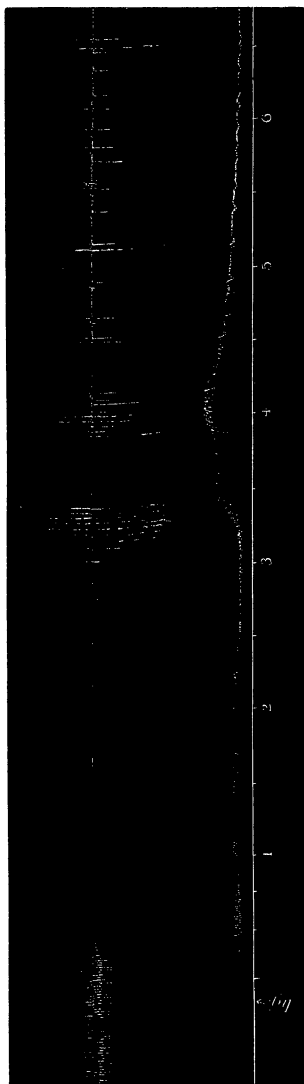
Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	145	55	
After $\frac{1}{2}$ min. . .	55	27	Marked fall of blood pressure.
" 1 " . . .	55	3	Respirations stopped.
After 2 mins. . .	58	0	
" 3 " . . .	40	1	
" 4 " . . .	115	13	Convulsive respirations.
" 5 " . . .	65	11	Convulsive respirations.
" 6 " . . .	50	7	Convulsive respirations.
" 8 " . . .	25	2	Convulsive respirations.
" 12 " . . .	20	14	Convulsive respirations.
" 14 " . . .	15	0	Respirations finally ceased.

(See Tracing VIII.)

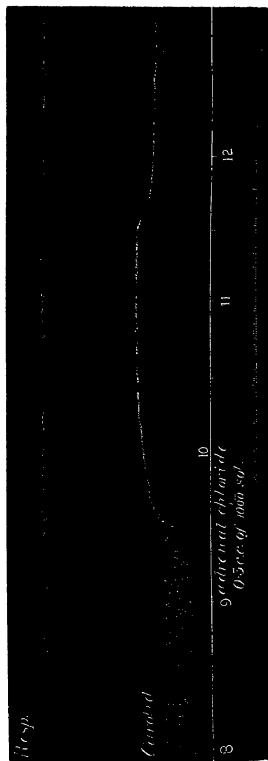
Before the injection of the venom the abdomen was opened, and a piece of omentum protected by cotton-wool soaked in warm normal salt solution was placed under the microscope and the circulation watched. Coincidentally with the first fall in the blood pressure marked dilatation of the portal vessels was observed to take place, and in a few minutes red corpuscles were seen to be exuding through the walls of the small vessels and capillaries, and before the conclusion of the experiment small petechial hæmorrhages were visible both in the part of the membrane under the microscope and also in parts freshly withdrawn from the abdominal cavity.

Post-mortem.—No clotting was found in either the portal, pulmonary or systemic circulations or in the heart. The blood was fluid and incoagulable. Very marked petechial hæmorrhages were found in the peri- and endo-cardium, and in all parts of the peritoneum, but especially in the mesentery and omentum. A smaller number were also found in the pleura. The brain and medulla were carefully examined, but no hæmorrhages were found in them. The whole of the portal system was greatly congested, as it always is in cases of Viperine poisoning.

This experiment is a very interesting one, as it affords complete proof of marked vaso-dilatation of the portal system coincidentally with the sudden fall in blood



Tracing VIII. (Crotalus, Experiment IV).—Cat. Crotalus venom. 5 cg. per kilogramme, intravenously.



Tracing IX. (Crotalus, Experiment V).—Cat. Crotalus venom. 1 cg. per kilogramme, intravenously. 8-13 minutes. Showing effect of adrenal extract.

pressure. Further it also shows the effect of respiratory convulsions in raising the blood pressure again, just as we found to occur in Daboia poisoning, although in the above experiment this effect was only temporary on account of the large dose of venom used. The tracing also well shows the continuance of the pulse after the sudden fall of pressure, so that this could not have been due to stopping of the heart, while there was absolutely no intravascular clotting to produce it. The cessation of respiration was doubtless due to the sudden deprivation of the medulla of blood by its being so rapidly drained into the capacious portal system. In the case of Experiments I. and II., in which smaller doses were used, the portal dilatation was less rapidly complete, and consequently the respiratory centre was able to hold out longer. As cats are much better than rabbits for studying blood pressure effects, the above experiment was repeated with a smaller dose.

EXPERIMENT V.—A Cat, weighing 3·2 kg., was chloroformed, and 1 cm. per kilogramme in 1 cub. centim. salt solution was injected intravenously, the respirations and the carotid pressure being accorded as in the previous experiments, and the effect of adrenal extract also tried.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	110	44	
After $\frac{1}{2}$ min. . .	75	20	Marked fall of blood pressure.
" 1 " . .	40	8	Respiration stopped.
" 2 mins. . .	15	0	
" 4 " . .	20	14	Respiratory convulsions.
" 5 " . .	95	13	Marked rise of pressure.
" 6 " . .	80	7	
" 7 " . .	65	20	Inspiratory gasps.
" 8 " . .	50-75	9	Traube-Hering curves well marked.
" 9 " . .	50-75	9	Traube-Hering curves well marked.
Adrenal chloride (1 in 1000) 0·3 cub. centim. in 1 cub. centim. salt solution injected intravenously.			
After 10 mins. . .	125	4	Marked rise in pressure. Respirations very shallow.
" 11 " . .	140	1	
" 15 " . .	115	9	
" 23 " . .	100	9	
" 27 " . .	55-100	6	Traube-Hering curves reappearing.
" 33 " . .	120	—	Respiratory convulsions.
" 35 " . .	70-100	6	
" 37 " . .	70-75	6	
" 41 " . .	60-70	—	Deep inspiratory gasps.
" 44 " . .	30	6	Respirations very shallow.
" 45 " . .	70	—	Respiratory convulsions.
" 50 " . .	40	—	Respiratory convulsions.
" 52 " . .	70	—	Respiratory convulsions. Central end of sciatic nerve stimulated.
" 54 " . .	50	—	Central end of sciatic nerve stimulated, no effect.
" 58 " . .	30	—	Respiratory convulsions feebler.
" 60 " . .	20	—	Respiratory convulsions ceased.

(See Tracing IX. for 9-13 minutes.)

Post-mortem.—The heart, portal, pulmonary and systemic circulations were free from clot, and the blood was incoagulable. There were extensive petechial hæmorrhages in the peri- and endo-cardium, the peritoneum and the pleura. The phrenic nerves were not paralysed.

This experiment presents several points of interest. The same circulatory failure and cessation of respiration, followed by respiratory convulsions and pumping-up again of the blood pressure, through the action of the diaphragm and other muscles on the dilated portal vessels, occurred as in Experiment IV. The dose, however, being smaller, the blood pressure recovered to a greater extent, and the Traube-Hering curves, indicative of the struggles of the partially paralysed vaso-motor centre, were well marked. At this point a single intravenous injection of a small dose of adrenal chloride produced a most marked effect on the blood pressure, raising it to above the original height before the injection of the poison. This effect was very much more lasting than in previous cases in which the drug was given after the paralysis of the vaso-motor centre had become complete, for in the present case a good pressure was maintained for 25 minutes, and it was not until the lapse of 40 minutes that the pressure again fell to the same point as before the injection. It is evident from this that much good may be expected to result from the use of this drug in cases of poisoning by the bites of Vipers by maintaining the circulation sufficiently to prevent the fatal secondary respiratory failure from want of blood supply to the medulla. Lastly, the slight rise of pressure on stimulation of the central end of the sciatic nerve after 52 minutes, and its failure to evoke any rise by this means, shortly before death, indicates that the complete paralysis of this centre was the cause of the final circulatory failure and the secondary cessation of respiration.

In order to demonstrate the dilatation of the portal vessels, coincidently with the fall in blood pressure due to the Rattlesnake venom, the following experiment was carried out.

EXPERIMENT VI.—A Cat, weighing 3 kg., was chloroformed and 1 cg. per kilogramme of Rattlesnake venom injected intravenously, a loop of small intestine having first been placed in an oncometer.

Time.	Blood pressure.	Oncometer lever.	Remarks.
	millims.		
Before injection .	150	Level.	
After $\frac{1}{2}$ min. . .	130-150	Rising.	Vaso-dilatation.
" 1 " . . .	130	Rising.	Vaso-dilatation.
" $1\frac{1}{2}$ mins. .	90-120	Slight fall.	Passive contraction.
" 2 " . . .	100-120	Rising.	Vaso-dilatation.
" 4 " . . .	105	Rising.	Vaso-dilatation.
" 6 " . . .	90	Rising.	Vaso-dilatation.
" 9 " . . .	85	Rising.	Vaso-dilatation.
" 12 " . . .	75	Rising.	Vaso-dilatation.
" 14 " . . .	75	Level.	

EXPERIMENT VI.—*continued.*

Time.	Blood pressure.	Oncometer lever.	Remarks.
	millims.		
2nd injection of 1 cc. per kilogramme in 0.5 cub. centim. salt solution.			
After 15 mins. . .	60	Very slight fall.	Respiratory convulsions, passive dilatation.
" 16 " . . .	55	Rising slowly.	
" 18 " . . .	75	Rising.	
" 21 " . . .	60	Rising.	

By this time the pulsation of the oncometer lever had ceased.

Post-mortem, the blood was incoagulable, and no clotting could be found anywhere. Hæmorrhages were found as usual in the heart and peritoneum. The phrenic nerves were not paralysed.

In this experiment slight falls of the oncometer lever occurred for a very short time shortly after each injection, pointing to a momentary weakening of the heart as the injection passed through it. With this exception there was evidence of steady vaso-dilatation of the portal vessels occurring with the steady fall of pressure in the general circulation. Strong solutions of Rattlesnake venom, such as 2–10 per cent., I have found to cause temporary weakening of the contraction of excised frogs' hearts, while the doses used (1 cc. in 0.5 cub. centims.) were injected in a 2-per-cent. solution. This effect on the heart passes off, as the venom becomes immediately diluted in the blood stream, and the subsequent steady fall of pressure is due to vaso-dilatation, as shown by the marked rise of the oncometer lever in the above experiment. WEIR MITCHELL and REICHERT (9) also observed some effect of Rattlesnake venom in producing vaso-motor paralysis in their classical experiments, but they considered that the venom had a very marked effect in paralysing the respiration. If the latter is the main cause of death then artificial respiration ought to prolong life and assist the circulation, as it does in the case of the pure Colubrine snake poisons. In one experiment with Rattlesnake venom in a rabbit, in which the respirations appeared to be affected in a more marked manner than usual, I tried the effect of artificial respiration with the following result.

EXPERIMENT VII.—A Rabbit, weighing 1·75 kg., was chloroformed and 3·5 cg. (2 cg. per kilogramme) injected intravenously in 0·35 cub. centim. salt solution.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	50	86	
After 1 min. . .	50	83	
" 2 mins. . .	40	85	Fall in blood pressure.
" 3 " . .	35	68	
" 4 " . .	30	60	
" 6 " . .	30	30	Respirations ceased. Artificial respiration started.
" 7 " . .	25	—	Artificial respiration.
" 10 " . .	25	—	Respiratory convulsions.
" 11 " . .	25	—	Artificial respiration.
" 12 " . .	20	—	Artificial respiration.
" 13 " . .	15	—	Artificial respiration. No effect.

Post-mortem, no clots could be found anywhere. The phrenic nerves were not paralysed. No good effect was produced by the artificial respiration, while the fact that the phrenic nerves were unaffected showed that no respiratory paralysis of a similar nature to that produced by pure Colubrine poisons had occurred.

II. *Trimeresurus anamallensis*.

Only three tracings illustrating the action of this venom have been obtained, but, read in the light of the results got with the other Viperine poisons, they suffice to afford a good idea of the action of this venom.

EXPERIMENT I.—A Rabbit, weighing 1½ kg., was chloroformed and 5 mg. per kilogramme (in 0·75 cub. centim. salt solution) was injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Remarks.
	millims.		
Before injection . .	40	108	
After ½ min. . .	25	—	Marked fall in blood pressure.
" 1 " . .	25	145	Respirations quickened.
" 2 mins. . .	25	134	
" 3 " . .	25	99	
" 4 " . .	20	93	
" 5 " . .	20	83	Respirations slowed.
" 6 " . .	15	59	
" 8 " . .	15	24	
" 10 " . .	10	0	Respirations ceased.

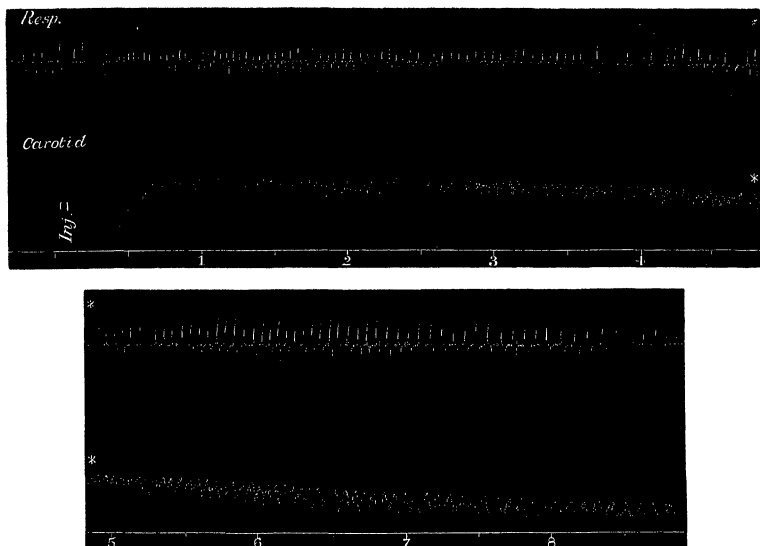
Post-mortem.—The phrenics were not paralysed, stimulation with a weak faradic current (secondary coil at 45 millims.) producing a good contraction of the diaphragm. The heart was beating when the chest was opened, and a very small clot was found in the right auricle. A small clot, $\frac{1}{2}$ inch in length, was found in the external jugular vein immediately below the point of injection, but no other trace of clotting could be found in either the portal, pulmonary or systemic circulations, including the heart. The blood when placed in a test-tube clotted very slowly and feebly, and on the following day some slightly hæmoglobin-stained serum had exuded, showing some hæmolytic action of the venom. The vessels of the portal system were greatly congested.

Here we have the same primary circulatory failure, followed by a slower failure of respiration, that we found in the case of the Rattlesnake. The stimulation of the respiratory centre in the early part of the experiment points to a direct effect of the venom on the respiration, and it is impossible to say how far the succeeding slowing down and stopping of the breathing was due to the direct action of the poison on the respiratory centre, and how far it was secondary to the failure of the respiration. The absence of any paralysis of the phrenic nerve proves that the respiratory failure was not of the same nature as that produced by Colubrine poisons, so that it appears to be more likely that the cessation of the breathing was purely brought about by deprivation of the medulla of sufficient blood by reason of the circulatory failure.

EXPERIMENT II.—A Cat, weighing 3 kg., was chloroformed and injected intravenously with 1.5 mg. (5 mg. per kilogramme) of *Trimeresurus* venom in 0.5 cub. centim. of salt solution.

Time.	Blood pressure.	Respirations per minute.	Remarks.
Before injection	millims. 140	16	
After $\frac{1}{2}$ min.	60	—	Rapid fall in pressure.
" 1 "	100	21	Partial recovery of pressure.
" 2 mins.	100	21	Respirations increased.
" 3 "	80-110	19	Traube-Hering curves appearing.
" 4 "	75-100	17	
" 6 "	60-80	17	
" 8 "	50-65	12	Respirations decreased.
" 10 "	50-60	8	
" 12 "	57	4	
" 15 "	85	—	Infrequent respiratory gasps.
" 18 "	65	—	Infrequent respiratory gasps.
" 20 "	100	—	Frequent respiratory convulsions.
" 24 "	70-90	9	Traube-Hering curves reappearing.
" 28 "	55-70	10	Infrequent deep respirations.
" 34 "	50-65	8	Infrequent deep respirations.
" 44 "	50-65	8	Infrequent deep respirations.
" 55 "	50-62	11	
" 60 "	52-65	13	Pressure and respirations improving.
" 65 "	55-70	16	Pressure and respirations improving.

(See Tracing X. for the 7th minute and the 16th to 23rd minutes.)



Tracing X. (*Trimeresurus anamallensis*, Experiment II.).—Cut. *Trimeresurus* venom, 5 mg. per kilogramme, intravenously.

At this point recovery was beginning to take place, the pupils, which had been widely dilated, were beginning to contract, and both the blood pressure and the frequency of the respirations were improving, so the animal was killed.

Post-mortem.—The phrenic nerves were not paralysed. The blood from the portal vein was incoagulable and after 24 hours the corpuscles had subsided, leaving slightly blood-stained fluid serum in the upper layers. The blood from the venæ cavæ clotted feebly, and after 24 hours had given out a small quantity of slightly blood-stained serum. No trace of clotting could be found in either the portal, pulmonary or systemic circulations or in the heart. There were a few small pericardial hæmorrhages on the surface of the heart, and also well-marked endocardial petechiæ in the left ventricle, but none could be found in the mesentery or omentum. These hæmorrhages were much less marked than that found in poisoning by the Rattlesnake or Puff Adder, but slightly more marked than *Daboia* venom causes.

This experiment is a very instructive one. The first sudden fall of blood pressure may possibly have been partly cardiac in origin, but the subsequent steady fall with well marked Traube-Hering curves was undoubtedly due to vaso-motor paralysis, as the tracing in all respects resembles those produced by the other Viperine poisons, which we have seen clearly cause vaso-motor paralysis. The pumping-up of the

blood pressure on the occurrence of respiratory convulsions was well shown in the curve. It is also of great interest to note that the Traube-Hering curves persisted throughout, and at the end of the experiment the animal showed evident signs of recovery, for we have already seen that as long as these curves remain the vaso-motor centre is not completely paralysed, for stimulation of the central end of the sciatic nerve causes a rise in the blood pressure, while drugs such as adrenal extract, which raise the pressure, have a much more lasting effect in this stage.

It is evident, then, that as long as the vaso-motor centre is not completely paralysed recovery may take place, and in borderland cases any treatment which raises the blood pressure sufficiently to keep the respiratory centre going will be of great service. The entire absence of intravascular clotting in this experiment is similar to the absence of that feature in poisoning by the other *Crotalidæ* examined, namely, the Rattlesnake. The respiratory failure in this case appears to have been entirely secondary to the failure of the circulation, for with the first signs of improvement in the latter the frequency of the respirations also increased. In the next experiment a larger dose was used, and a loop of the small intestine was also placed in an oncometer.

EXPERIMENT III.—A Cat, weighing 2 kg., was chloroformed, and after a loop of small intestine had been placed in an oncometer, 1 eg. per kilogramme in 0.5 cub. centim. salt solution was injected intravenously.

Time.	Blood pressure.	Respirations per minute.	Oncometer lever.	Remarks.
Before injection . .	millims. 100	18	Rising slowly	
After $\frac{1}{2}$ min. . .	15	7	Sudden marked fall	
„ 3 mins. . .	25	—	Final respiratory convulsions	

(See Tracing XI.)

Post-mortem.—The phrenic nerves were not paralysed. There was extensive intravascular clotting throughout the portal circulation, the pulmonary arteries, the systemic veins, and the right side of the heart. The remaining blood clotted very feebly, and after 24 hours exuded a little blood-stained serum.

Here we have a typical death due to intravascular clotting, and it is interesting to observe the sudden fall of pressure in the portal circulation with the general fall of blood pressure, that is just the opposite to what occurs in Viperine poisoning without any intravascular clotting. We also have here one of the *Crotalidæ* producing in a large intravenous dose clotting in the blood vessels precisely similar to that produced by the pure Vipers, but which I have never obtained with the other Pit Viper, namely,

the Rattlesnake, except at the seat of injection of the venom. On the other hand, the *Trimeresurus* has much less effect than the Rattlesnake in producing hæmorrhages, in which respect it resembles the *Daboia* more closely than any of the other Vipers dealt with in this paper.

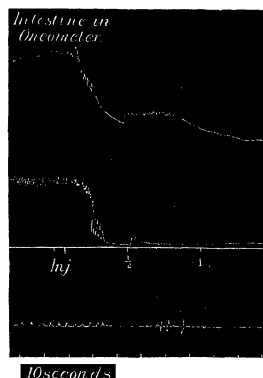
Comparison and Summary of the Action of the Vipers and Pit Vipers.

A comparison of the results obtained with the four Viperine poisons dealt with above shows a most striking general resemblance together with most marked differences in degree, more especially with regard to the effect on the coagulability of the blood as shown by intravascular clotting. This can be best brought out by comparing the effect of the different poisons on the various systems.

I. Action on the Blood.

1. *On the Coagulability.*—The action of Viperine poisons in producing intravascular clotting is the most striking factor met with. Yet we find it differs very greatly in degree in the different venoms examined. That of the *Daboia Russellii* nearly always kills the comparative small animals used by producing intravascular clotting of the blood whenever a single lethal dose is given either subcutaneously or intravascularly, the symptoms being of an acute and rapidly fatal character. The Puff Adder venom also in a single lethal dose either subcutaneously in pigeons, or intravenously in larger animals, produces intravascular clotting, but in proportion to its minimal lethal dose in pigeons it requires a larger dose of it to cause clotting than of *Daboia* venom. Next, passing on to the Pit Vipers we find that in the case of *Trimeresurus anamallensis* fatal failure of the circulation can be produced by a single small intravenous dose without any intravascular clotting other than locally in the vein through which the venom enters the circulation. Yet a larger dose may produce marked and rapidly fatal intravascular clotting as in Experiment III. with this venom. Lastly, in the case of the Rattlesnake venom I have never found any intravascular clotting, except once at the seat of injection, after death brought about by a single intravenous injection of the venom. These facts alone are sufficient to show that intravascular clotting is not an essential lethal action of the Viperine snakes as a class.

But we may go further, for the experiments recorded in this paper show that in



Tracing XI (*T. anamallensis*, Experiment III.).—Cat. *Trimeresurus* venom. 1 cg. per kilogramme, intravenously. Intestine tracing above, carotid below.

the case of all these venoms, by giving small preliminary doses either subcutaneously or intravenously, so as to bring about the negative phase of reduced coagulability, death due to circulatory failure can be readily produced without the occurrence of any intravascular clotting. The blood in such cases will remain fluid or clot only very feebly after death when placed in test-tubes. There is evidently, then, some important lethal action other than the intravascular clotting of the blood to be considered.

2. *Hæmolytic Action*.—In all of the four Viperine venoms dealt with there is evidence of fairly well-marked hæmolytic action, for if the fluid blood be allowed to stand in a test-tube for 24 hours the red corpuscles will settle, and the upper layers will consist of hæmoglobin-stained serum. LAMB (12) has recently studied this effect and concludes that the hæmolytic action of Daboia venom is more marked *in vivo* than *in vitro*, while in the case of Cobra venom the reverse holds good. He regards the fact that the blood-stained discharges in Daboia poisoning contain few red corpuscles as evidence of marked hæmolytic action, but this must be largely due to the effusion of serum brought about by the vaso-motor paralysis, which I have found to be the most essential and constant action of Viperine poisons, and to their effect in dissolving the walls of the small vessels, which we have next to discuss.

3. *Hæmorrhagic Effects*.—In the records of the experiments recorded in this paper we have repeatedly come across marked petechial hæmorrhages, especially in the mesentery and omentum, and in the peri- and endo-cardium, as well as less frequently in the pleura. Here, again, we find great differences in degree in the case of the several Viperine poisons examined. Thus the greatest hæmorrhagic effects are undoubtedly produced by the Rattlesnake poison, for in Experiment IV., although the experiment only lasted 15 minutes, yet there were marked hæmorrhages at the end of that time. FLEXNER has recently studied this phenomenon and attributes it to a dissolution of the endothelial cells lining the small blood vessels and capillaries, which would largely account for the extreme rapidity of the process noted above, although I think the vaso-dilatation produced by all these venoms must materially aid it, for the changes are most marked in the distribution of the portal circulation, where the vaso-motor paralysis is most felt. The other Pit Viper, the *Trimeresurus*, has a much less marked action in this direction, although it is still a very definite one. On the other hand, the true Viper, the Puff Adder, has a very marked hæmorrhagic action, more marked indeed than the *Trimeresurus*, so that this property is in no way peculiar to the Pit Vipers. Lastly, the Daboia has a very slight action in producing these petechial hæmorrhages compared to that of the Rattlesnake or Puff Adder, yet endocardial petechiæ in the left ventricle result from its action in prolonged cases, so that the difference is one rather of degree than of kind, all of the Vipers yet examined showing it to some extent. Nevertheless the lethal effects of these poisons, at any rate in the case of the comparatively rapid deaths here dealt with, cannot be attributed to this hæmorrhagic action, although they doubtless play a large part in

the more chronic forms produced by slightly *supra*-minimal lethal doses such as are likely to be met with in man.

It has hitherto been thought that the fatal effects in man produced by Viperine poisons are due to the loss of coagulability of the blood and consequent hæmorrhages, but it is not quite clear how such fluidity of the blood can bring about a rapidly fatal termination, seeing that the coagulability may be enormously reduced in hæmophilia without any such rapid death with hæmorrhages, from the bowel more particularly, taking place as in Viperine poisoning. Further, we have seen that in the case of each of the venoms dealt with a rapidly fatal failure of the circulation can be produced without any intravascular clotting, and without sufficient hæmorrhages to in any way account for the fatal termination, and the factor which produces this must also be operative in the more chronic cases with more marked hæmorrhages. Further, we know from the researches of WEIR MITCHELL (9) that complete loss of coagulability of the blood, with very marked hæmorrhages from the bowel and other parts, of several days' duration, may yet be completely recovered from, while some of the cases recorded by FAYRER (2) of bites by Indian Vipers, including one by a *Trimeresurus*, presented similar remarkable recoveries. It is very difficult to account for these on the theory that the whole of the pathology of Viperine poisoning is summed up in the blood changes dealt with above.

If, on the other hand, there is in addition a more powerful circulatory effect produced by the poison, which is the real cause of the lethal results, and this condition may be recovered from after it has persisted for some time, the explanation will become much easier. In the case of intravenous injections of poisons not only will the symptoms be rapidly produced, but if the dose is insufficient to produce death, then recovery will be proportionally rapid. More than one example of this occurred in the experiments recorded in this paper. Take, for example, the Experiment II. with the *Trimeresurus* venom, in which at the end of an hour both recovery of the circulation and increased frequency of the respirations began to take place, yet on killing the animal the blood was completely incoagulable (no clotting taking place in 24 hours in a test-tube), while hæmorrhages had commenced to take place both in the peritoneum and in the heart. Here the commencing recovery was clearly due to improvement in the circulatory conditions in spite of the persistence of the blood alterations. Other similar instances might be given, but enough has been said to prove that the circulatory changes referred to afford a more satisfactory explanation of the lethal effects of these venoms than do the blood changes taken by themselves.

II. *The Circulatory Effects of the Venoms.*

1. *Action on the Heart.*—The first three venoms have been applied in solutions of varying strengths, directly to excised frogs' hearts, and the contractions recorded. In the case of *Daboia* venom, solutions as strong as any used in the injections did not

retard the contractions of the heart. In that of the Puff Adder temporary stimulation was produced by strong solutions, while with Rattlesnake venom in very strong solutions the contractions of the heart were somewhat weakened for a short period, but in no case was it rapidly stopped. It is possible that a small part of the rapid circulatory failure may be due to a direct effect of the Rattlesnake venom in its passage through the heart, but it would immediately become diluted in the blood stream to such an extent as to lose this effect, while the continuance of the heart beats for long periods shows conclusively that the circulatory failure cannot be due to failure of the heart's action.

2. *Paralysis of the Central Vaso-Motor Centre.*—As the circulatory failure cannot be explained as due to any direct action of the venom on the heart itself, we must consider how far an effect on the vaso-motor mechanism will explain the facts recorded. Capillary clotting having been excluded by transfusion of the portal, pulmonary, and systemic circulations after death having demonstrated that there was no obstruction in them; we find that a paralysis of the central vaso-motor centre will fully account for all the phenomena met with. Thus, the increased excursus of the pulse, so constantly seen in the tracings, indicates diminished peripheral resistance; the pumping-up of the fallen blood pressure on the occurrence of respiratory convulsive efforts, which has formed such a remarkable feature of some of the charts of each of the venoms dealt with, can only be explained as the result of a mechanical aid to the circulation brought about by pressure on the dilated portal circulation, while the good effect of abdominal pressure is also accounted for on the same hypothesis; the remarkable Traube-Hering curves so frequently met with, and persisting as long as the central vaso-motor centre is not too completely paralysed to respond to strong stimulation of the central end of the sciatic nerve; the extreme portal dilatation met with after death, and the rapid dilatation of the vessels of the omentum and mesentery coincidently with the primary fall in pressure, which has been observed with the microscope in several instances; and lastly, and most conclusive of all, the demonstration of the portal dilatation by means of a large loop of small intestine in an oncometer, at the very time that the pressure in the general circulation is declining; all prove that the essential action common to all the four Viperine poisons dealt with is a vaso-motor paralysis, which is undoubtedly of central origin, for the action of Daboia venom on the vessels themselves has been found to be one of vaso-constriction, which can also be brought about by the action of such drugs as adrenal extract and nicotine after the vaso-motor centre itself is completely paralysed.

If the dose of venom given intravenously is sufficient to paralyse the vaso-motor centre very rapidly the circulatory failure will be too quick to be recovered from even temporarily. On the other hand, if a smaller dose be given, then the portal dilatation is more gradual, and time is given for the circulation to accommodate itself to the altered conditions sufficiently to allow of enough blood being sent to the

medulla to enable it to keep up infrequent and often convulsive respirations, accompanied by well-marked Traube-Hering curves. Close observation now shows the sequence of events to be as follows: A deep inspiration is taken, and the breath is held, during which time the blood pressure reaches and is maintained at the height of the curve. Then an expiration follows, and immediately the pressure gives way a little, to rise once more with the next inspiration. As the vaso-motor centre becomes more and more enfeebled these curves become less marked, and by the time the centre no longer responds to stimulation of the central end of the sciatic nerve they have disappeared, the centre being now completely paralysed, although adrenal extract will still produce a temporary rise of pressure by contracting the peripheral vessels. The respirations now grow less and less frequent, and are of a convulsive nature, and then finally cease, and then death takes place.

If, on the other hand, the dose is still less, and the vaso-motor centre begins to recover its tone, the blood pressure rises slowly once more, and coincidentally the respirations improve in frequency, and recovery takes place in spite of complete incoagulability of the blood, and well-marked visceral hæmorrhages. All the facts recorded can, then, be explained as a result of paralysis of the central vaso-motor centre, and in no other way.

III. *Action on the Respiratory Centre.*

The failure of respiration occurring in the case of Viperine poisons can best be explained as being secondary to the failure of the circulation brought about by the central vaso-motor paralysis. Whether they have any direct action on the respiratory centre as well, as held by WEIR MITCHELL and REICHELT and by FLEXNER in the case of the Rattlesnake, is open to doubt. In favour of such an action is the fact that in the cases in which small doses of Daboia venom were given, to induce the negative phase of reduced coagulability, the number of respirations were nearly always reduced very considerably before any marked fall of blood pressure had taken place. Further, in some instances when a second dose of venom was injected, after complete vaso-motor paralysis had taken place, the already much reduced respirations speedily failed altogether and death took place, as, for example, in the fourth Puff Adder experiment. In one experiment (not mentioned hitherto) two small subcutaneous doses of Daboia venom, which had been mixed for $\frac{1}{2}$ hour with CALMETTE's antivenin, were not followed by the usual slowing of the respiration, which would seem to point to a small amount of Colubrine poison present having been neutralised by the serum. On the other hand is the fact that in no case of Viperine poisoning was there any evidence of paralysis of the end-plates of the phrenic nerves. The evidence does not seem to be sufficient to decide the point either way, and it must be left to further experiments, on the lines of C. J. MARTIN's method of separating the Colubrine from the Viperine element by filtering through gelatine under pressure, to determine it.

Antidotes to Viperine Venoms.

If my conclusion is correct that the most essential lethal action of the Viperine poisons as a class is a paralysis of the central vaso-motor centre, much as that of the Colubrine class is a paralysis of the respiratory centre, then it would appear to be probable that an antivenin which would be effective against the vipers might be made on the same lines as CALMETTE'S serum, which I have shown in the first part of this paper has a very definite, if not very powerful, action against all the purely Colubrine poisons tested. In support of this view it may be urged that D. D. CUNNINGHAM working in Calcutta did succeed in protecting a goat against a fatal dose of Daboia venom by means of repeated subcutaneous injections of this venom, so that it would be well worth while to attempt to make such a serum as that suggested.

Until, however, such a serum is prepared we must content ourselves with trying to counteract the physiological action of the Viperine poisons by such measures as appear likely to serve this purpose. The most promising of these which have been found to improve the blood pressure in my experiments are, firstly, mechanical pressure such as a binder applied to the abdomen, to which might be added bandages applied to the limbs so as to conserve as much blood as possible for the urgent needs of maintaining the circulation through the brain and medulla, and, secondly, such drugs as contract the peripheral vessels, of which the most powerful are adrenal extract and nicotinic. The former in particular has a most beneficial and lasting effect, so long as the vaso-motor centre is not completely paralysed, as in Experiment V. with the Rattlesnake poison. In borderland cases there is good reason to hope that some lives may be saved by such rational lines of treatment based on the presence of vaso-motor paresis.

In conclusion, I have to express my great indebtedness to the Senate of the London University, for permission to work in their Physiological Laboratory, and to Dr. A. D. WALLER, Dr. T. G. BRODIE and Dr. N. H. ALCOCK for much very valuable advice and help in the course of the investigation.

REFERENCES.

1. "On the Physiological Action of the Poison of the Hydrophidæ," 'Proceedings of the Royal Society,' 1903, vol. 71.
2. Sir JOSEPH FAYRER'S 'Thanatophidia of India,' 1872. Churchill, London.
3. WALL'S 'Indian Snake-poisons, their nature and effect,' W. H. Allen & Co., London, 1883.

4. C. J. MARTIN, 'Proceedings of the Royal Society of New South Wales,' July 3rd, 1895.
5. LAMB and HANNA, 'Journal of Pathology and Bacteriology,' 1902, vol. 8, p. 1.
6. BRUNTON and FAYRER, 'Proceedings of the Royal Society,' 1873-4.
7. C. J. MARTIN and CHERRY, 'British Medical Journal,' 1898, p. 1120; 'Proceedings, Royal Society,' vol. 63, 1898, p. 420.
8. LAMB and HANNA, 'Lancet,' June 15, 1901.
9. WEIR MITCHELL and REICHERT, 'Smithsonian Contributions to Knowledge,' vol. 26, 1890.
10. FLEXNER and NOGUCHI, 'University of Pennsylvania Medical Bulletin,' 1902, p. 345, and 'Journal of Pathology and Bacteriology,' 1903.
11. D. D. CUNNINGHAM, 'Scientific Memoirs by Medical Officers of the Indian Army,' Part IX., 1895, and Part XI., 1898.
12. LAMB, 'Scientific Memoirs by Medical Officers of the Indian Army,' No. 4, 1903.

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Phil. Trans., B, vol. 197, 1904, pp. 193-247

VII. *On the Structure of the Palaeozoic Seed* *Lagenostoma* Lomaxi, with a Statement of the Evidence upon which it is referred to *Lyginodendron*.

By F. W. OLIVER, M.A., D.Sc., Quain Professor of Botany in University College, London, and D. H. SCOTT, M.A., Ph.D., F.R.S., Honorary Keeper of the Jodrell Laboratory, Royal Gardens, Kew.

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[PLATES 4–10.]

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INTRODUCTION.

IN a note communicated to the Royal Society in May, 1903,* it was stated that a re-examination of the Palæozoic seeds, placed by WILLIAMSON in his genus

* 'Roy. Soc. Proc.,' vol. 71, p. 477.

Lagenostoma, had revealed unexpected points of agreement between the structure of the envelopes of certain of these seeds on the one hand and that of the vegetative organs of *Lyginodendron* on the other, and the conclusion was drawn that the seed *Lagenostoma Lomaxi* could have belonged to no other plant than *Lyginodendron*.

In the present paper it is proposed to give a full statement of the evidence upon which that conclusion is based, to describe the seed in detail, and to consider the bearing of the discovery upon the question of the relations of the Ferns and Gymnosperms.

Two species of *Lagenostoma* (*L. ovoides* and *L. physoides*) were described by WILLIAMSON in his eighth memoir "On the Organisation of the Fossil Plants of the Coal Measures";* a third species which he named in his MS. catalogue, *L. Lomaxi*, after its discoverer, was left undescribed by him.

This seed (*L. Lomaxi*) is occasionally found attached to its pedicel, and is further remarkable as compared with other members of the genus in being found enclosed in an envelope or cupule springing from the pedicel just below the base of the seed and extending above the micropyle, at least in young specimens. The cupule appears to have been ribbed below, and deeply lobed in its upper part; in form it may be roughly compared to the husk of a hazel nut, of course on a very small scale.†

The pedicel and cupule bear numerous capitate glands, which, though in a state of disorganisation, have been found to offer the closest agreement in size, form, and structure, with the glands which occur on the vegetative organs of *Lyginodendron Oldhamium*, and which are especially abundant in the particular form of that plant found in association with *Lagenostoma Lomaxi*.

The reference of the seed to *Lyginodendron* is primarily based on this agreement in gland structure, but as the sequel shows, a comparison of the detailed anatomical structure of the cupule and pedicel further supports the attribution.

In the present paper we deal in successive sections: first, with the structure of the seed and with its pollination; then follows the description of the cupule and stalk, and a comparison of their structure with that of the vegetative organs of *Lyginodendron*; this includes a statement of the evidence on which we base the reference of the seed. Finally, there are sections dealing with the position of the seed on the plant, with the morphology of the seed and cupule, and with the systematic position of the genus.

THE SEED.

The seeds which have come under our observation fall under three categories. First, there are the full-sized specimens with dimensions averaging $5\frac{1}{2} \times 4\frac{1}{2}$ millims.;

* 'Phil Trans,' vol. 167, p. 233, figs. 53-75 and 77-79, 1877.

† The photograph on p. 217 represents a restoration in wax of one of the smaller seeds, enclosed in its cupule. The model was prepared for the authors by Mr. H. E. H. SMEDLEY. The ripe nut of *Corylus*

these form the great majority. Then there are occasional smaller seeds, which, though immature in some respects, are from the appearance of their membranes and state of preservation to be regarded rather as undersized abortive seeds than as real ovular stages, that is to say, like little acorns that prematurely fall, they have reached their full limits of development. Only four seeds of this kind have passed through our hands, and of these, two which are cut across the apex lack the enveloping cupule.

Finally, there is a solitary specimen of a small seed enclosed in the cupule, which, from the peculiarity of its preservation, has considerable claim to rank as a real ovule, an immature stage, that is, possessing capacity for further development.

In the course of this memoir we enumerate all the more important specimens of *Lagenostoma Lomaxi* and of its stalk and cupule, which have been consulted in the course of the investigation. In this connection our thanks are due to Miss M. BENSON of the Royal Holloway College, to Professor C. E. BERTRAND of the University of Lille, to Mr. R. KIDSTON, F.R.S., and to Professors F. E. WEISS and I. BAYLEY BALFOUR, F.R.S., all of whom have freely placed at our disposition fossil slides in their custody or possession.

The specimens upon which our description of the full-grown seed is based are as follows :—

- | | |
|---|--|
| * WILLIAMSON Collection, 1915, M. | } Series of three transverse sections from |
| * " " " 1915, O. | |
| * SCOTT Collection, 236 | |
| U. C. L. Collection, R. 6, a | } Series of three transverse sections from |
| * " " " R. 6, b | |
| * " " " R. 6, c | |
| U. C. L. Collection, R. 7, a | } Series of two transverse oblique sections |
| * " " " R. 7, b | |
| * U. C. L. Collection, R. 8, a | } Series of two transverse sections from |
| * " " " R. 8, b | |
| U. C. L. Collection; R. 9, a | } Series of three transverse sections of the |
| " " " R. 9, b | |
| * " " " R. 9, c | |
| * Collection of Botanical Dept., Univ. of | } Series of three oblique sections from the |
| Lille, 1110 | |
| * " " " 1108 | |
| " " " 1109 | } Series of two oblique sections through |
| WILLIAMSON Collection, 1931 | |
| * KIDSTON Collection, 260 | |

Colurna, L., enclosed in its glandular husk, may be cited as affording a remarkably accurate model of our seed in its cupule, so far as the form and general relations of the parts are concerned.

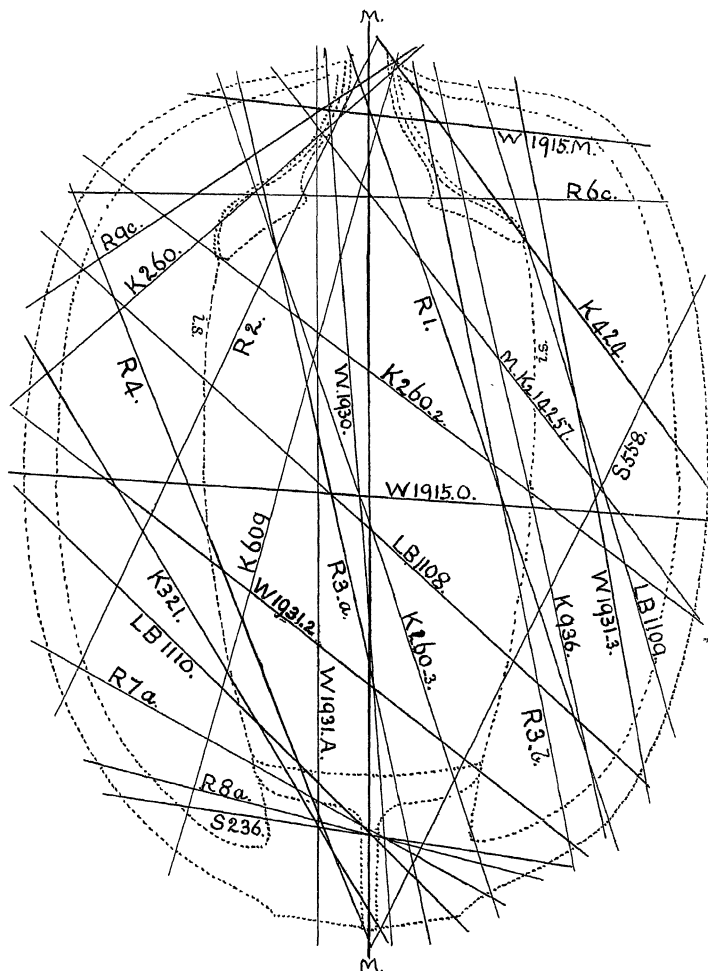
KIDSTON Collection, 321	}	Series of two oblique sections through the same seed.
* " " 424		
WILLIAMSON Collection, 1915 G	}	Series of two oblique sections through the same seed.
" " 1915 R		
* U. C. L. Collection, R. 3, <i>a</i>	}	Series of two longitudinal sections through the same seed.
" " R. 3, <i>b</i>		
* WILLIAMSON Collection, 1930		Longitudinal section.
" " 1931, A		" "
* U. C. L. Collection, R. 1		Longitudinal section, showing pollen-chamber.
* " " R. 2		Longitudinal section, showing pollen-chamber.
* SCOTT Collection, 558		Longitudinal section, showing attachment to pedicel.
* U. C. L. Collection, R. 4.		Longitudinal section, showing base.
* Manchester Museum Collection, K ₂ 14257.		Oblique section, showing pollen-chamber.
KIDSTON Collection, 609		Longitudinal section, showing pollen-chamber.
U. C. L. Collection, R. 10		Oblique section of canopy.
KIDSTON Collection, 936		Longitudinal section.
" " 937		" "

The preparations preceded by a * are figured in the present paper.

The Full-Sized Seed.

Although the sections of full-sized seeds examined have been plentiful, not one is a truly median longitudinal section. Very few approach the median plane so nearly as to traverse both the pollen-chamber and the scar at the chalaza where the seed was attached. The majority of "longitudinal sections" cut the median plane by 15° or more, so that the real relations of a median section have been obtained by a process of building up oblique sections of various sorts. In this way, with the aid of improvised models and diagrams, an ideal median section has been reached, the proof of whose approximate accuracy depends on the fact that the various regions in any given oblique section fall into their right position in regard to it.

The accompanying diagram (text-fig. 1) represents the true median section of the seed, and upon it have been plotted the approximate planes of all the more important sections that have passed through our hands, including those referred to in the present memoir. The diagram is in no sense a restoration, the displaced internal tissues are represented occupying the position in which they are usually



TEXT-FIG. 1.—Diagrammatic Median Longitudinal Section of the Seed *Lagenostoma Lomaxi*, upon which are plotted the approximate positions of the planes of section of most of the important sections figured or cited in this memoir. The reference letters and numbers given with each section on the figure are the designations under which the preparations are cited in the text and in the explanation to the plates. M., M., Median plane; i.s., intermediate sack. W. 1930 should be somewhat nearer the median plane. The small seed in W. 1931A (phot. 1) is not plotted; the section is almost median.

W = WILLIAMSON Collection. M = MANCHESTER MUSEUM Collection.
 K = KIDSTON Collection. LB = UNIVERSITY OF LILLE, BOTANICAL DEPARTMENT Collection.
 S = SCOTT Collection. R = UNIVERSITY COLLEGE, LONDON, BOTANICAL DEPARTMENT Collection.

found in the fossils. It exhibits many of the salient features of the seed, which conforms to the Cycadean type in its general relations. The height somewhat exceeds the diameter, and its figure is that of a prolate spheroid. The full-sized specimens were about $5\frac{1}{2}$ millims. high, and had a diameter of $4\frac{1}{4}$ millims. at the equator. The central body of the seed, the nucellus, is free from the integument in the apical region only for a distance equal to about one-fifth the height of the seed. It consists of a conical or slightly pyramidal pollen-chamber resting on a broad convex plinth, which overlies the chamber of the megaspore. Around the free part of the nucellus is a massive and rather complicated integument (here termed the canopy) which barely reaches up to the tip of the pollen-chamber, the latter actually protruding beyond the micropyle.

Below the level of separation, integument and nucellus become confluent as in *Cycas*. The wall of the seed throughout is a simple shell consisting of a few layers of elongated fibrous elements limited externally by a well-marked palisade layer, the cells of which bear short peg-like processes. The interior tissue has separated from the wall of the convex sides of the seed in nearly all cases, and hangs suspended like a sack between the pollen-chamber and chalaza. The long gaps shown to the right and left of the intermediate sack in many longitudinal sections (text-fig. 1; and Plate 4, phot. 5; Plate 5, phot. 9) correspond to the peripheral space that has arisen during the decay of the seed. Within the suspended sack the much-contracted megaspore is usually visible, containing traces of prothallial tissue (phot. 5 and fig. 20).

The vascular system of the seed is derived from a single supply bundle, which shortly after its entry at the base of the seed branches, as a rule, into nine radiating strands. In the majority of specimens these strands run in a plane immediately exterior to the intermediate sack of the seed (text-fig. 1, and Plate 7, fig. 2), though their original position was much closer to the surface. At the apex they enter the several loculi of the canopy and end close to the micropyle.

Around the micropyle the surface of the seed shows nine radially running ridges which die out almost at once. Though standing out in low relief, these ridges form very conspicuous features in oblique sections through the apex when their apparent height becomes much exaggerated, as in the specimen U. C. L., R. 9, c (Plate 5, phot. 10). These ridges are the only external mark of one of the peculiar features of the seed, the radial partitioning of the canopy into nine symmetrically disposed and connivent loculi. Just as the stigmatic bands overlie the placentas of a poppy-capsule, so here are the ridges superposed to the septa of the canopy.

The general relations of the seed, including all the features touched on above, find admirable illustration in the series of three transverse sections from one seed (W. 1915, M.; 1915, O.; S. 236) represented in Plate 7, figs. 1, 2, and 3. This series has a further interest in that the seed, although of full size, was still enclosed in the remains of the cupule. The approximate positions of the three sections are indicated in the diagram (text-fig. 1).

The lowest section (fig. 3) is across the chalazal region just below the plane in which the main vascular bundle breaks into its nine radiating strands. The eccentric position of the fibrous cushion which surrounds the vascular bundle arises from the fact that the section is not truly at right angles to the axis of the seed, an explanation holding good in the other two members of the series as well. The section across the canopy (fig. 1) shows the nine loculi—the radial septa becoming incomplete on that side of the section which dips to a slightly lower level. Within the canopy, with cusps corresponding to its folds, lies the wall of the pollen-chamber, and inside this, like a little circular island, is the transversely cut central cone.

The outside wall of both these sections is very thick in comparison with its true diameter (as shown by the middle section, fig. 2), owing to the nearly tangential plane in which the wall has been cut. In both sections, but particularly in that across the canopy (fig. 1), many series of palisade cells are cut through before the section has done with this layer. And we note the further point that the palisade cells stand in radial files, a result of the meridian-like lines in which the palisade cells are doubtless distributed over the surface. This conclusion finds corroboration from a study of the chalazal section (fig. 3, *part.*).

The middle section of the series (fig. 2) comes from the broadest part of the seed. It shows the intermediate sack and crumpled megaspore wall, and, just outside the former, the ring of lenticular tracheal strands. The section is a thoroughly typical one across the middle of a seed. The seed seems to have been generally detached through the agency of a layer of separation formed right across the seed-base at its junction with the pedicel (Plate 10, fig. 26), and all sections appropriately directed in the chalazal region traverse the circular scar below which the abscission-layer was developed. Only a single specimen of a full-sized seed still attached to its stalk has come under our observation (S. 558, fig. 33), though the seed of which the three transverse sections are figured (figs. 1, 2, and 3) may very likely be another instance, as the remains of the cupule are still in position. Unfortunately a study of a section from the same block cut below the seed has been without decisive result (see p. 221, footnote).

In passing to the detailed description of the various parts of the full-sized seed, the following order is observed :—

(a) The nucellus, with sections dealing with the pollen-chamber, the pollen, and the intermediate sack and megaspore.

(b) The integument, including its free and confluent portions, the chalaza and the vascular system.

(a) THE NUCELLUS.

The Pollen-Chamber.

The summit of the free portion of the nucellus is modified to form the pollen-chamber. In form it is bottle-shaped, with the neck just protruding from the

micropyle (Plate 4, photos. 1, 2 and 6, Plate 9, fig. 21). At its insertion upon the convex plinth it undergoes a slight constriction (phot. 6 and fig. 19). The upper third of the sloping surface is bevelled in correspondence with the convexities of the canopy, suggesting that the angles of the former engaged with the grooves of the latter, like a nut in a spanner. This bevelling is shown in all transverse and oblique sections of this part of the pollen-chamber (figs. 20, 22 and 23).

Towards the base of the chamber the bevelling dies out, and the sinus between the pollen-chamber wall and the canopy sensibly broadens in the peripheral direction into a sort of cloister running all round the plinth on which the pollen-chamber stands, and limited externally by the receding slope of the canopy (phot. 6, *s.*, fig. 20, *s.*).

In the small abortive seeds the sinus is not continued below the base of the pollen-chamber (W. 1931, A. small seed, phot. 2 and fig. 24, *s.*), as the supporting plinth, no doubt a result of intercalary stretching, had not as yet developed.

The wall of the pollen-chamber is unusually well preserved and was evidently of robust construction. It consists of a single layer of cells, the elements of which have somewhat oblique end-walls (fig. 21, *p.c.w.*). A conspicuous feature of this layer is the relatively great radial thickness of the cells, especially in the lower region of the pollen-chamber, where their tangential walls often project as convex papillae (figs. 19 and 24). Distally the layer thins out considerably, and in the region of the neck, where it is in close contact with the canopy, it has almost a membranous thinness and is usually much darkened by carbonisation (figs. 19 and 21).

The contour of the chamber varies in different sections according to their plane. The only really longitudinal section, that shown in W. 1931 A. (see phot. 2 and fig. 24), shows it to have been rather truncate in form like a water bottle.

The pear-shaped form seen in U. C. L., R.1 (fig. 19), and the still more tapering outline in U. C. L., R.2 (phot. 8 and fig. 21), both depend on the fact that these sections are oblique (*cf.* text-fig. 1, where the planes of section are plotted). The former cuts the base of the pollen-chamber midway between the centre and the periphery, whilst the latter follows a plane parallel to and close within the sloping wall, which it intersects just above the level of the insertion of the pollen-chamber upon the plinth.

A still more oblique section is shown by the Manchester specimen, K₂, 14257 (fig. 20). This intersects the base at an angle of 45° and then travels out of the pollen-chamber about half-way up the sloping wall. The bevelling of the wall is excellently shown in this specimen. Yet another oblique section of the pollen-chamber is shown in a specimen from the KIDSTON Collection, No. 260 (fig. 22).

The interior of the pollen-chamber is occupied by a cone of delicate tissue which stands erect from the floor. On the flanks this cone is not in continuity with the wall, from which it is separated by a cleft, narrow above but broadening below (*p.c.*, phot. 2 and figs. 19, 21, 24). This space has, no doubt, arisen by the separation of the epidermis from the subjacent tissue. This cleft is the actual pollen-chamber

and it is in its lower third that pollen-grains occur. It is bell-shaped in form and communicates with the exterior at the apex by a circular slit (o, figs. 19 and 21).

The tissue composing the central cone is a homogeneous, thin-walled parenchyma, the cells of which are arranged in longitudinal series (figs. 19 and 24, cc.). At the base of the chamber the peripheral layers of the cone are continuous with the wall, but elsewhere, both on the flanks and at the base, the tissues of the central cone end abruptly (figs. 19 and 21). Indications of a continuity with the nucellar tissues that survive in the intermediate sack of the seed are not wanting, but of course in full-sized seeds the megaspore had advanced right into the plinth, so that by its subsequent contraction on the death of the seed comparatively little tissue can have been destroyed (phot. 2).

The cone as a whole, no doubt, represents the apex of the nucellus from which the epidermis has become separated to form the cleft-like pollen-chamber.

The Pollen.

Only four sections of full-sized seeds traversing the lower part of the pollen-chamber have come under observation. Of these the one from Stalybridge (U. C. L., R. 2) contains traces of about six pollen-grains (Plate 5, phot. 8 and Plate 9, fig. 21, p.g., p.g.), whilst in a preparation from another seed (U. C. L., R. 1, fig. 19) two are present. In the Manchester section (K₃, 14257) there is a single pollen-grain (fig. 20, p.g.), whilst in W. 1930 there is no trace of pollen, although an object is present which, though suggesting a pollen-grain at first sight, is doubtless of fungal origin (phot. 6, to the right of the pollen-chamber, low down).*

The pollen-grains are oval in form and have average dimensions of $70 \times 55\mu$. Indications of an internal cellular reticulum are sometimes shown (fig. 21, p.g.), but the state of preservation of the pollen in this species, so far as it has come under our observation, hardly justifies a detailed description. It is much inferior to *Lagenostoma ovoides* in this respect.

No pollen is present in any of the small-sized seeds.

The Intermediate Sack and Megaspore.

The sack-like envelope which stretches from the edge of the plinth below the pollen-chamber to the well-preserved chalazal plate, consists of the collapsed layers that have separated from the shell previous to fossilisation (photos. 7 and 9, i.s.).

The separation has usually occurred in the hypoderm of the shell some three or four layers down from the palisade layer, and probably corresponds to the plane in which the softer internal tissues abutted on the harder layer of the integument.

* We have found in other specimens many bodies probably referable to Fungi, both in the palisade layer of the testa and elsewhere.

In the displaced tissues, which morphologically, no doubt, include portions of both integument and nucellus, the most conspicuous feature is the ring of vascular bundles held together tangentially in a thin brown layer of collapsed and discoloured cells (see the specimen W. 1915, O., fig. 2, *i.s.*). This layer has an irregular stratified structure and sometimes shows a tendency to split into two concentric shells (as in U. C. L., R. 6 *b*, fig. 31, *b.r.* and *i.r.*), of which the outer contains the vascular strands, whilst the inner forms the actual pocket containing the megaspore.

Occasionally an additional detached ring is found outside the bundle-ring (as in U. C. L., R. 9 *b*), but on the whole the mode of splitting of the peripheral tissues is remarkably uniform, giving either a simple intermediate sack (as in fig. 2), or a special bundle-ring with a partly separated inner sack, as in fig. 31).

The original position of these layers is rarely preserved, but in the transverse section across the middle of a seed, U. C. L., R. 7, *b*, (represented in fig. 29), the bundles (*v.b.*) are evidently still *in situ*, though indications of a tendency to split are manifest both within and without the bundle-ring.

The intermediate sack merges below into the well-preserved tissues of the nucellar base (W. 1930, phot. 5 and 7; U. C. L., R. 3, *a*, phot. 9), whilst above it is attached to the trough of the sinus. An inner layer of tissue passes beneath the plinth, and was no doubt continuous with the tissue of the central cone of the pollen-chamber (W. 1931, A. phot. 2). The outer shell, containing the tracheal strands, works round the angle of the sinus and enters the canopy, where it either rests against the inner fluted wall (W. 1930, phot. 6), or more frequently passes upward lying freely in the loculus (U. C. L., R. 3, *a*, phot. 9). These soft internal tissues of the seed are usually much collapsed and distorted in full-sized seeds.

The single megaspore has contracted away from the interior sack in almost all cases, its thick structureless membrane being thrown into numerous plaitings and corrugations (*e.g.*, W. 1930, M.; Manchester, K₂, 14257; U. C. L., R. 3, *a*, *cf.* phot. 5, fig. 20, *m.g.*, and phot. 9). Its figure is very irregular and in a majority of cases it has retreated into the broader upper region of the central cavity (shown in W. 1930, phot. 5). It often contains traces of prothallial tissue (as in U. C. L., R. 3, *a*, phot. 9), but no indications of archegonia or of an embryo have been recognised.

(b.) THE INTEGUMENT.

Having dealt with the nucellar region of the seed we pass on to the description of its integument and peripheral regions generally. The free part of the integument, the canopy, may be taken first, whilst in succeeding paragraphs the palisade layer, the chalaza, and the vascular system are in turn described.

The Canopy.

This name may be conveniently applied to the whole of the complicated free portion of the integument which forms the hollow, fluted dome around the plinth and pollen-chamber. Its essential peculiarity depends on the fact that it is not a mere sclerotic wall but a series of tangentially joined structures of considerable radial extent and differentiation. The outer surface of the canopy entirely resembles that of the rest of the seed, and there is no external mark indicating the actual zone at which the integument becomes free. Its lining layer, however, is fluted and closely invests the upper portion of the pollen-chamber. It springs from the margin of the plinth, which is slightly cusped, so that even at its actual insertion incipient furrows are already present on this lining layer. That the plinth has an angled contour may be inferred from many longitudinal sections, *e.g.*, U. C. L., R. 1.; W. 1930 (*cf.* fig. 19 and phot. 6), in which its halves are asymmetrical and unequal. That its convex surface bore low undulations is seen in the section U. C. L., R. 3, α (phot. 9), where the two obtuse upwardly directed angles of the plinth correspond with the folds in the canopy above.

Before elucidating the structure of the canopy by a consideration of the various sections, it may be explained that the sinus between pollen-chamber and canopy is a conspicuous feature in most sections from this part of the seed, and that in full-sized specimens it formed a roomy cloister below and around the base of the pollen-chamber.

To consider first the transverse sections of the canopy. The lowest of these, cut as nearly as possible horizontally at the level of the base of the pollen-chamber (*cf.* text-fig. 1), is shown in the specimen U. C. L., R. 6, *c.* The section is limited externally by the palisade layer (fig. 30, *pal.*) and underlying hypoderm. Following a space of .5 millim. (fig. 30, *o.s.*), comes a second concentric shell (*b.r.*), and then a third (*i.t.*). The second and third shells are angled, and the outer of the two contains the vascular bundles midway between its angles. The latter is the upper continuation of the ordinary bundle-ring so well shown in the transverse section W. 1915, O. (fig. 2, *i.s.*). The inmost shell is the indurated inside wall of the integument already showing the commencement of the grooving which becomes so conspicuous higher up. The pollen-chamber is not represented as the section is incomplete.

The next section is cut half-way up the pollen-chamber and is from one of the small abortive seeds (U. C. L., R. 11). Externally it is limited by the immature palisade layer upon which about several layers of hypoderm (fig. 23). From this hypodermal zone spring three arcs, each consisting of a double row of cells. The filling tissue of these arcs has not been preserved and their actual insertions upon the hypoderm are ill-defined for the same reason. Nearer the centre of the figure is the angled wall of the pollen-chamber (*p.c.w.*), evidently displaced.

The deeply grooved layer which the series of arcs collectively form, no doubt corresponds with the inmost shell of the specimen U. C. L., R. 6 α (fig. 30, *i.t.*) with its cusps, of course at a higher level (fig. 23, *i.t.*). Although the preservation leaves something to be desired, there is little doubt that the lining layer (*i.t.*), maintains its individuality as it rounds the successive grooves. The points of this layer still show traces of continuity with the peripheral tissue lying below the palisade cells, though in full-sized seeds, in which the softer tissues are rarely preserved, the continuity would be broken and the grooves would lie quite free from the peripheral layer.

The highest of the horizontal sections through the canopy that we have examined is the upper member of the series of three (W. 1915, M., Plate 7, fig. 1). The full number of nine loculi is represented in the canopy. The centre of the section is occupied by the slightly angled pollen-chamber (*p.c.*), and this is surrounded by the fluted, inner wall of the integument (*i.t.*). Of the septa which lie between the loculi, five reach the outer wall, whilst the remaining four fall short. The loculi alternating with these imperfect septa have larger cavities than have those which are completely septate. The explanation is, of course, that the section is slightly tilted so that the chambers on one side are cut at a slightly lower level than on the other (*cf.* plotting of W. 1215, M. in text-fig. 1, p. 197). Those with imperfect septa are cut relatively low down—at a level corresponding to R. 11 (fig. 23)—the others at a higher level where septation is complete, *i.e.*, at which the indurated lining of the furrows and peripheral wall are continuous.

The configuration of the lining of the canopy is somewhat as follows: At its insertion upon the edge of the plinth the organ shows the beginnings of nine radial grooves which deepen as the micropyle is approached. Alternating with these grooves are an equal number of projecting cushions, the convexities of which are directed towards the axis of the seed. This folded organ, resembling an umbrella being opened against a gust of wind, is the internal boundary of the free integument, and by the junction of its upper deeply folded part with the outside wall gives rise to the characteristic "chambers" of this part of the seed. But even after the chambers are complete the double nature of the septa can still be detected. This is shown in W. 1915, M. (Plate 7, fig. 1), also in the oblique Manchester K₂ 14257 (Plate 9, fig. 20), and in the still more oblique KIDSTON 424 (fig. 25). In U. C. L., R. 9, *c* (Plate 5, phot. 10), where the septa are perfectly double, the appearance depends on the fact that they are cut almost longitudinally and not through the narrowest parts of the grooves (for the position of this section *cf.* text-fig. 1, U. C. L., R. 9, *c*).

The relation of the canopy to the sinus between it and the pollen-chamber is well illustrated by a reference to phot. 9, a longitudinal and slightly oblique section through the canopy. The plane of section cuts the plinth midway between its margin and the base of the pollen-chamber. It cuts the chamber of the canopy about its axis almost at right angles, and of course the gaping portions of the two grooves, being cut at right angles, are seen at a minimum. The walls of the left-hand

furrow fail to reach the outside wall, demonstrating how short is the distance from the apex to which the distal ends of the complete septa extend. Though the lower part of the canopy hangs well above the plinth, leaving a roomy cloister, higher up this sinus becomes narrower and chink-like. Indeed the convexities of the canopy probably engaged with the bevelled facets of the upper part of the pollen-chamber wall. This is shown by the transverse section across the canopy in W. 1915, M. (fig. 1), and also by the oblique Manchester section K_s. 14257 (fig. 20), which traverses the base and side of the pollen-chamber, and further by the even more oblique KIDSTON 260, which cuts the upper part of the pollen-chamber at a very steep angle (fig. 22). The detached island of tissue (*i.e.*), in this figure, below the tangentially cut pollen-chamber, is a portion of the sagging wall of the loculus of the canopy which lies in the plane of the section (*cf.* text-fig. 1, K. 260).

The grooving of the interior of the canopy is represented on the outside wall of the seed by the little ridges which radiate from the micropyle, running in the same radial planes as the septa. These ridges are seen in every section that cuts the top of the seed, and in the case of very oblique sections their height is much exaggerated. The true height of the ridges is given by the Manchester section K_s. 14257 (fig. 20, *r*), which cuts the surface near the apex at right angles. Here at a distance of .3 millim. from the micropyle the height of the ridge is 170 μ . In the specimen U. C. L., R. 3, *a* (phot. 9), from a section cutting the ovule $\frac{1}{2}$ millim. from the micropyle, the ridge has practically died out, so that the radial extension of these ridges was trifling. It is in the oblique slices from the top of the seed, such as U. C. L., R. 9, *c*; K. 260 and K. 421, represented in phot. 10, fig. 22 and fig. 25 (*r*) respectively, that the ridges are shown to such advantage, sections in which the ridges are cut very obliquely indeed. The planes in which these sections are cut are shown in text-fig. 1. In Manchester K_s. 14257 (fig. 20) the ridges overlies septa which are cut in the same section; in fig. 22 and phot. 10 the ridges shown belong to the part of the canopy not exposed in the section.

The Palisade Layer.

The surface of the testa is invested everywhere, from the micropyle to the place of insertion of the seed on its pedicel, by a well-marked layer of closely fitting prismatic cells set at right angles to the surface and having a height of 150 μ and diameter of 60–80 μ (well shown in U. C. L., R. 3, *a*, phot. 11). As the separating zone at the seed-base is approached, the layer gradually dies out (*cf.* U. C. L., R. 4, fig. 26, and S. 558, fig. 33). The appearance of the palisade layer, when viewed in superficial tangential sections of the seed, shows that the palisade cells were arranged in longitudinal series, running from pole to pole like circles of longitude (W. 1915, M., fig. 1, *pal.*).

The manner of preservation of the cells varies from seed to seed. In a majority

of cases the walls are relatively thin and carbonised, the cell-contents being relatively inconspicuous (U. C. L., R. 3, *a*, phot. 11). In others, the cavities are filled with dense brown contents, and the walls have a lighter tint (U. C. L., R. 1 and 2, figs. 19 and 21, *pal.*); finally, the walls may be brown and very thick, a type of preservation which, though very usual in the allied species *L. ovoides*, is extremely rare in *L. Lomaxii*.

In most specimens the palisade cells bear little pegs at the surface, not always inserted centrally, and having a height reaching 20–24 μ and occasionally even 30 μ . Commonly these pegs are black and structureless (as in the Lille specimen 1108, fig. 28 A., and U. C. L., R. 3, *a*, phot. 11, *pe.*), whilst occasionally the body of the peg appears to be enclosed in a little cap of membrane (as in U. C. L., R. 9, *a* and *b*, and KIDSTON, 321, not figured). The ordinary type of peg (phot. 11 and fig. 28 A.), therefore, may be regarded as representing the carbonised and persistent contents of little cells exterior to the palisade layer.

In the small abortive seeds the palisade layer is not differentiated. Such seeds are limited by a layer of small cubical cells which bear peg-like papillæ, apparently not yet separated by transverse walls. It may be supposed that at a later stage of development this layer divided tangentially into two layers, of which the inner elongates to form the prismatic layer, whilst the outer survives in the form of pegs.

In the immediate neighbourhood of the micropyle the cells of the testa project as well-marked papillæ (W. 1931, A., fig. 32). These papillæ are not isolated quite in the same way as the pegs just described, but probably correspond to the distal ends of the mother-cells with which they remain, perhaps, in open connection.

The knob-like preservation of the surface layer, found in the young seed U. C. L., R. 13 (*cf.* p. 213), perhaps owes its origin to the entire contents of the layer of mother-cells being preserved in much the same way as are the pegs on full-sized specimens (*cf.* fig. 34, *pal.*).

There is a further peculiarity of the palisade layer deserving mention, in which the pegs seem to play a passive rôle. The latter are sometimes found raised up on tapering cones (as in the Lille specimen 1108), as though some mucilaginous substance had been emitted from the prismatic cells below, or from the wall upon which the pegs are seated (fig. 28 B.). In extreme cases the black pegs are raised to a considerable height in this way (U. C. L., 8, *b*, phot. 12, *pe.*). This phenomenon, as we find it in these seeds, was a local effect, and resulted in a blister-like raising of the organic *débris* by which the seed was closely invested (phot. 12, *mu.*, in which the mucilage cones are clearly shown). But although, perhaps, having their origin in some local lesion, it seems not improbable that the blisters observed by us are merely a sporadic and premature manifestation of a phenomenon which normally became general only at a latter stage in the history of the seed. This mechanism suggests comparison with the mucilaginous testas of many recent Angiospermic seeds.

The Chalaza.

This region of the seed is characterised by the relatively large amount of sclerotic tissue that is present at the seed-base. The thin shell of the seed expands abruptly to form a funnel-shaped cushion or cup, in the cavity of which the base of the nucellus rests (well shown in the median section W. 1930, phot. 7).

The chalaza is sharply defined below by the layer of separation which completes the surface of the adult seed where it was attached to its stalk (phot. 7, *a.l.*). The only full-sized specimen still remaining on its pedicel (S. 558, phot. 15 and fig. 33) shows the layer of separation (*a.l.*) cutting across the tissues in such a way as to leave a low projecting cushion on the seed when it became detached. And all the other seeds cut in an appropriate plane agree in showing a convex cicatrix. When the section cuts the scar in the median plane (as in phot. 7) the cushion is shown to be conical, but most sections across the cushion miss the tapering apex. The surface of the papilla is covered with about two layers of small iso-diametric cells (U. C. L., R. 4, fig. 26), in connection with which the severance took place.

Just within the papilla and occupying its whole width is the funnel-shaped sclerotic cushion. The elements which compose it are long and fibrous; they have fairly thick walls, and the contents are more or less blackened. At first these fibres run parallel to the long axis of the seed, but soon they diverge like a brush and the peripherally placed fibres take a superficial course below the palisade of the testa and form the hypoderm of the shell throughout the seed (U. C. L., R. 4, fig. 26, *hy.*). The interior fibres diverge less sharply and form a thick and closely knit sheath symmetrically disposed around the axial supply-bundle on its entry from the pedicel (*cf.* the median sections W. 1931 A. and W. 1930, photos. 3 and 7, *ch.c.*). The sclerotic character of these cells ceases abruptly at a distance of $\frac{1}{2}$ – $\frac{2}{3}$ millim. from the scar, and their further continuation is at best represented for a short distance by a more or less collapsed reticulum of loose strands which corresponds to the insertion of the softer peripheral tissues of the seed-base.

The hollow in the sclerotic cushion has the form of a champagne glass (phot. 7, *v.l.*), and as the bowl expands the entering bundle separates into the peripheral vascular strands, which run in the plane of the glass.

Such sections as are not median, but cut the chalazal papilla between its periphery and the centre and then run up obliquely in a direction *away from* the axis of the seed (*cf.* text-fig. 1, S. 558; U. C. L., R. 4), pass more or less tangentially along the sclerotic cushion, so that this structure is shown with a convex upper boundary (see the specimens S. 558, and U. C. L., R. 4, figs. 33 and 26, *ch.c.*). Again, in other sections which start like the last, but take an oblique direction *towards* the axis of the seed (text-fig. 1, Lille 1110), the relation of the parts must vary according to the plane followed. The section of the Lille specimen, 1110 (fig. 27), intersects the bundle at its place of branching and almost coincides with the limiting slope of

the bundle-socket on the distal side (*cf.* text-fig. 1). Thus, two of the branch-bundles are exposed in longitudinal section for some distance (fig. 27, *b* and *c*), and the section eventually traverses the corner of the intermediate sack or megaspore pocket, of course cutting others of the vascular strands *d*, *e*, *f* higher up as they pass towards the apex. The specimen U. C. L., R. 7, *a* (not figured) is a section following an almost identical plane (*cf.* text-fig. 1, p. 197).

Transverse sections near the base of the seed, such, for instance, as the lowest of the series of three, S. 236 (fig. 3) and U. C. L., R. 8, *a* (phot. 13), show the chalazal cushion as an annular zone of transversely cut fibres with the supply bundle (*v.b.*) occupying the centre. Around the cushion is a gap bridged at places by the shreds of looser tissue, whilst peripherally the seed is closed in by the hypoderm and palisade layer. The multi-seriate appearance of the last-named is an obvious consequence of the almost tangential plane in which the testa is cut.

The eccentricity of the several zones in both these sections depends, of course, on the fact that the plane of section is not truly transverse (*cf.* text-fig. 1). In this part of the seed even very slight changes in the angle of cutting lead to marked alterations in the distribution of the various zones.

Overlying the sclerotic cushion is a somewhat concave plate (W. 1930, and U. C. L., R. 3, *a*, fig. 5 and phot. 9, *nu.*) forming the bed upon which the base of the intermediate sack rests. It is loosely compacted of thick-walled oval cells, whose longer axes usually run in the radial direction. Thus, in the specimens U. C. L., R. 4 and R. 3, *a* (fig. 26 and phot. 9), these elements are cut nearly transversely in the middle parts of the section, in the Lille specimen 1110 (fig. 27, *nu.*) almost longitudinally.

Peripherally the edges of the bed or plate bend up and become continuous with the sides of the intermediate sack.

The Vascular System of the Seed.

The bundles that traverse the seed form a conspicuous feature in almost every preparation, alike of older and younger specimens.

The vascular supply is derived from the chalazal bundle that enters at the seed-base by its subdivision into a whorl of nine bundles, which range themselves symmetrically around the intermediate sack and pass distally into the loculi of the canopy.

The chalazal bundle follows the axis of the seed in its passage through the chalazal cushion. At a distance of $\frac{1}{2}$ – $\frac{3}{4}$ millim. from the scar at the seed-base it expands into a funnel (Plate 7, fig. 5) and breaks up into the nine peripheral bundles which at once bend sharply outwards and shape their course so as to round the base of the intermediate sack of the seed. The connection between the chalazal and peripheral bundles for each category of seed is well shown in the three median sections that are

figured: In the young seed or ovule, U. C. L., R. 13 (fig. 33, *a*); in the small abortive seed, W. 1931, A. (Plate 4, phot. 3); and in the full-sized specimen, W. 1930 (phot. 7 and fig. 5). The place of branching of the chalazal bundle is also cut in several of the oblique sections through this part of the seed, a type of section well illustrated by the Lille specimen 1110 (fig. 27). This figure shows three divergent bundles, *a*, *b* and *c*, of which *b* and *c* are cut nearly in longitudinal section. Other similar sections, such, for instance, as U. C. L., R. 7, *a*, are in essential agreement.

Of the chalazal bundle below its point of branching two sections are figured. The lowest member of the series of three sections of one seed (S. 236), illustrated generally in fig. 3, cuts this bundle transversely. An enlarged view of the same bundle is given in fig. 4, and a longitudinal section from W. 1931, A., in fig. 5. Another specimen, also cut transversely, is clearly indicated in U. C. L., R. 8, *a*, (phot. 13, *v.b.*).

The nine branch-bundles after rounding the base of the nucellus make their way upwards in most specimens in the collapsed layers of soft tissue which collectively form the intermediate sack of the seed. The oblique Lille section (1110, fig. 27) shows three of these bundles (*d*, *e*, *f*) shortly after turning the corner of the megaspore sack, whilst transverse sections across the middle of the seed (of which W. 1915, M., fig. 2 is a perfectly typical example) are characterised by a circle of nine bundles, withdrawn some distance from the periphery of the seed, and connected together by the intermediate sack. In longitudinal sections the number of bundles cut of course depends upon the obliquity of the section (*cf.* phot. 5, phot. 9, and fig. 20).

The true position of these bundles in the body of the seed is demonstrated by the section U. C. L., R. 7, *b* (fig. 29), where they are found *in situ*. The single bundle represented in this figure (*v.b.*) is still in continuity with the hypoderm (*hy.*), and is separated from the superficial palisade layer by about five layers of cells.

As the bundles approach the apex they pass outside the periphery of the plinth and enter the canopy, one to each loculus (figs. 1, 23, and 30). That the bundles cling very closely to the outside angle of the sinus (see phot. 6 and 9 and fig. 20) seems to be due to the fact that in this region, as in the body of the seed, they have suffered a centripetal displacement. No section, however, has passed through our hands in which the bundles at this height are seen lying *in situ*, though in the specimen U. C. L., R. 6, *c* (fig. 30, *v.b.*) their position is probably not far from that which they occupied in the living seed. That a single bundle normally entered each of the nine loculi of the canopy is shown by almost every section through this part of the seed (figs. 1, 20, 23, 25), though the bundles are not always preserved, especially in the distal parts of the loculi.

The form of the chalazal bundle in transverse section is roughly circular with dimensions for the xylem strand of $84 \times 70\mu$; the peripheral bundles, on the other hand, have an elliptical section (W. 1915, O., phot. 14), with average dimensions at

the mid-height of the seed ranging from $160-176\mu \times 60-70\mu$. The major axes of the bundles lie in the tangential plane.

In their passage to the apex of the seed the strands taper to some extent, but they retain the elliptical outline throughout.

In one instance a bundle was found to fork in passing from the chalaza to the canopy. The section of the central seed, of the row of three full-sized specimens in the preparation W. 1931, passes through the basal half and shows a ring of nine bundles. A second section higher up this same seed occurs in the KIDSTON preparation No. 260. In this section there is a ring of ten bundles. This is the only departure we have met with in *Lagenostoma Lomaxii* from the constant number of nine peripheral bundles. That this is a case of a bundle forking on its way up the seed rather than a fusion of two bundles depends, of course, on the correct determination of the relation of the two sections. In the absence of any trace of the chalazal tissues or of the canopy or pollen-chamber in either of the sections, the recognition of W. 1931 as the lower section is based on the relative diameter of the intermediate sack in the two sections in question. In W. 1931 the sack is 2 millims., and in KIDSTON 260 it is 3 millims. in diameter. As the intermediate sack is well known to expand like a funnel in its passage up the seed (e.g., specimen 1930, phot. 5), there is no doubt the smaller ring belongs to the lower section.*

The peripheral position of the bundles, as determined by the specimen U. C. L., R. 7, *b* (fig. 29), and the fact that they run into the free part of the integument or canopy, seems to stamp the bundles as definitely appertaining to the integument of the seed.

Turning now to the *structure* of the seed-bundles, it is mainly the xylem strands that are preserved. In the transversely cut chalazal bundle in the specimen, S. 236 (fig. 4), the xylem is shown with its smallest elements at the centre. In the slightly disorganised zone of soft tissue which accompanied the xylem strand it is not possible to differentiate the phloëm. The very similar section U. C. L., R. 8, *a* (phot. 13) shows the position of this zone, but it is much more disorganised.

The chalazal bundle is cut in longitudinal section in the specimen W. 1930 (phot. 7, *v.b.*). The enlarged drawing (fig. 5, *p.x.*) shows the small protoxylem elements to be centrally placed, thus confirming the indications given in the transverse section (S. 236, fig. 4). The chalazal strand is consequently mesarch.

In the branch-bundles, as seen in transverse section, the smallest xylem elements occur along the face directed towards the centre of the seed (W. 1915, O., phot. 14), or they may lie a little deeper in the strands (as in U. C. L., R. 8, *b*). The radial section of a bundle from a full-sized seed in W. 1931, A. (fig. 6) shows the sequence of the various elements. The protoxylem element (*p.x.*) lies close to the internal side of the strand, from which it is separated by another spiral element of slightly larger calibre. The other tracheides are spiral and scalariform, and in one case pitted. Similar indications are given by one of the bundles in KIDSTON 321. It

* The positions of the two sections are shown on the diagram, p. 197, at K 260-2 and W 1931-2.

thus appears that these peripheral strands are either endarch or very nearly so, the amount of centripetal xylem being, at the best, trifling. Some of the narrow elements shown accompanying the xylem in fig. 6 may be phloëm, but we are unable to speak confidently as to this part of the bundle as the indications are so slight.

In nearly all cases in which the structure of the peripheral bundles is shown in longitudinal section, and especially in those cases in which spiral elements have been recognised, it is in the lower part of their course. This is hardly to be explained as being due to the greater dimensions of the bundle below than above. It may perhaps be correlated with the fact that the upper part of the seed undergoes a relatively late extension. The only ground for this suggestion is the fact that the plinth is wanting in the small abortive seeds (phot. 2), so that in passing from the small to the full grown dimensions there must have been considerable intercalation in that particular zone of the seed.

The Abortive Seeds.

Besides the full-sized specimens, having approximate dimensions $5\frac{1}{2} \times 4\frac{1}{4}$ millims., there are the small, though in many respects mature-looking, seeds to which reference has already been made. Their dimensions are $2\frac{1}{2} \times 2$ millims.

Two of these smaller seeds have been found cut in longitudinal section, whilst in three other specimens parts of the canopy and pollen-chamber are shown.

The specimens are—

W. 1931, A., the smaller seed on this slide.

It is cut quite longitudinally and is still enveloped in its cupule (see phot. 1).

W. 1931, a small seed occurs at the edge of the section.

It is cut less medially than the last and like it is completely enveloped in its cupule (see phot. 4).

W. 1915 R., a transverse section across the canopy and pollen-chamber, with parts displaced.

U. C. L., R. 11, a section resembling the last, but nearer the apex.

U. C. L., R. 12, a median longitudinal section of pollen-chamber and canopy.

In their *form* these seeds differ slightly from the full-sized type.

If the small seed W. 1931, A. (phot. 1) be compared with the full-sized specimen W. 1930 (phot. 5), both being almost median sections, the former is found to be relatively stouter than the latter, i.e., the ratio of the transverse to the longitudinal diameter of the little seed is in excess of that of the full-sized seed. Further, the small seeds are somewhat more barrel-shaped than the large ones, their sides being relatively less convex.

The slight broadening towards the base shown by the specimen W. 1931, A. (see phot. 1) is probably the result of an injury which its chalaza sustained prior to

fossilisation, the chalazal cushion having been displaced some little distance into the interior of the seed.

As regards their *structure*, both the pollen-chamber and chalazal cushion of the small seeds are of full size, but the convex plinth which supports the former in the full-sized seed is here undeveloped. The sinus around the pollen-chamber is not continued beyond the base of the pollen-chamber. The plinth doubtless arose later by intercalary stretching in the zone below the pollen-chamber. The elongation referable to this cause alone corresponds to 20 per cent. of the total stretching that the seed must have undergone in passing from the arrested to the full dimensions.

The parenchyma flanking the central space is well preserved, particularly in the section of a small seed in W. 1931 (phot. 4), which, being cut somewhat tangentially lies in the plane of the intermediate sack. The vascular strands are completely differentiated, though they do not afford any special indications lacking in the larger seeds. The entering bundle in the small specimen W. 1931, A., as it expands at the base of the nucellus (phot. 3) is the counterpart of the strand as shown by the full-sized seed W. 1930 (phot. 1, *n.b.*).

The outer wall of the integument in small seeds presents certain peculiarities. The shell as a whole is thinner than in normal seeds (70μ as against 190μ), but its tissues do not appear to have been softer or less rigid than those of the mature specimens. The little seed in W. 1931, A. (phot. 1) has preserved its contour quite as fully as has the large seed in W. 1930 (phot. 5). The basal region perhaps may have been somewhat deficient in strength, as evidenced by the collapsed state of the chalaza in the former specimen. The tall palisade layer of the normal seed is represented here by a much smaller and nearly cubical cell-layer about one quarter the height (*cf.* phot. 11, and fig. 23, *pal.*). In both cases pegs are present on the surface, but whilst in the old seed the peg may reach a length of 25μ , in the abortive specimens they do not exceed $10\text{--}12\mu$ in height. Further, in the small seed the peg does not appear to be separated as a distinct cell, though in the normal condition it seems probable that such a separation occurred.

The most interesting features shown by these small seeds are: (1) The matured appearance of the tissues, especially of the shell, pollen-chamber and chalazal cushion; (2) the full size of the pollen-chamber, which, though it projects to the exterior, is without pollen-grains; (3) the absence of the plinth.

These specimens fell through the agency of the abscission-layer, the base of the seed being sharply isolated in both the complete examples (photos. 3 and 4, *a.l.*). The cupule, still closely investing the seed, fell with it, forcibly wrested from the pedicel after the separation of the seed.

The Small Seed or Ovule.

In addition to the abortive seeds, described in the last paragraph, another small specimen U. C. L., R. 13, has been examined which shows rather peculiar features,

The seed itself, which has a length of about 2 millims., is much altered and not easy of interpretation. The pollen-chamber, chalaza, and cupule alone are well-preserved, whilst the main body of the seed is much crumpled and the testa exhibits extreme decay. In all probability the specimen is a young or ovular stage with immature tissues. The section is longitudinal and median, and shows the seed and cupule both attached to the pedicel. The testa of the seed is represented by an extraordinary, irregular mass of dark strands, covered on the surface with black pegs, recalling those on the normal testa but longer (*cf.* fig. 34, *pal.*). The black pegs or knobs occur in all sorts of positions—not only towards the exterior. This is to be accounted for on the supposition that the testa is completely crumpled up and thrown into folds which the section cuts across at various angles. The knobs probably represent the entire contents of a disorganised mother-cell layer from which palisade layer and pegs normally develop.

The interior of the seed is occupied by three curious black-walled pipes (fig. 34, *i.s.*, *i.s.*) which run longitudinally—one above reaching up to the base of the central cone of the pollen-chamber, and two below, not far from the chalaza. These probably are the remains of the much-shrivelled nucellus and megaspore. Closely accompanying these structures, on either side and also between them, are the vascular bundles—an appearance due to the extreme shrivelling that the tissues have undergone. Throughout the specimen the vascular bundles are extremely well preserved. At the chalaza the branching of the main bundle is shown (fig. 34, *ch.*), and it can be traced right back into the pedicel where the insertion of one of the cupular bundles may be seen (*at a*). This is the only specimen we have met with showing the origination from the main vascular strand of both the cupular and peripheral seed-bundles. The cupule of this specimen is described below, p. 215.

POLLINATION.

A majority of the sections that traverse the lower part of the pollen-chamber of full-sized seeds afford evidence that pollination has been effected. The supposed ovule (U. C. L., R. 13, fig. 34), and the small arrested specimens, on the other hand, contain no pollen.

Nevertheless these young seeds were already provided with pollen-chambers of full size and mature appearance, whilst the orifice, also, projected well beyond the canopy (phot. 2 and fig. 24, *p.c'*).

These facts are consistent with the view that pollen was received by the seed while it was still fairly small; as soon, perhaps, as the unfolding of the cupule at the apex permitted. Nevertheless we hardly possess sufficient data for arriving at a decision as to the time of pollination. The little seeds seem ready for the pollen, but only full-sized specimens are known to contain pollen in the pollen-chambers. The reason, too, for the abortion of the small-sized seeds remains obscure. In view of

what is known to happen in the case of Cycads, it would be imprudent to conclude that abortion occurred from lack of pollination.

The full-sized pollinated seeds seem to have fallen through the agency of the abscission-layer, and but rarely to have remained involved in the cupule (as in the series represented in figs. 1, 2 and 3). That the specimen S. 558 (photos. 15 and 16) is still attached to its stalk was no doubt the result of accidental circumstances. The process of fertilisation probably took place on the ground. To an elucidation of the further history of the seed there are no data to guide us. It may be conjectured that it was not altogether unlike that of existing Cycads.

As regards the mode of pollination, how the pollen was brought and retained, just this can be said. There was in all probability a drop-mechanism at the tip of the seed by means of which the grains of pollen were retained and sucked or siphoned into the pollen-chamber, much as in recent Gymnosperms. Whether such a drop originated directly from the orifice of the pollen-chamber, or whether perhaps the tips of the loculi of the canopy may have co-operated in this service, must remain a matter for conjecture.* That the pollen was borne to the seed by the wind should not be tacitly assumed in the case of *Lagenostoma*. The protruding pollen-chamber and the presence of glands on the cupule suggest a possibility of the co-operation of insects.† Although the not very numerous available sections of the pollen-chamber of *L. Lomaxi* are inconclusive as to the amount of pollen that was delivered to this seed, the case is otherwise with the closely related *L. ovoides*. Here the evidence afforded by an extensive series of appropriate sections points to the existence of an unusually perfect mechanism for pollen-transfer. The pollen-chamber is, as a rule, abundantly provided with pollen-grains and sometimes it is almost choke-full. Taken in connection with the points already mentioned, the case of *L. ovoides* would seem to indicate a mechanism of pollen-transfer of greater precision than chance dispersal by wind. The question is one deserving further consideration, though we cannot pursue it here.

Summary for Seed.

Lagenostoma Lomaxi is an orthotropous seed of Cycadean organisation. It shows complete radial symmetry. Specimens of full size are about $5\frac{1}{2}$ millims. long and $4\frac{1}{4}$ millims. in diameter.

The central part of the seed is generally contracted away from the testa forming the intermediate sack in which the crumpled megaspore is contained.

The apex of the nucellus alone is free from the testa from which it protrudes. It

* Cf. 'Annals of Botany,' vol. 17, p. 462.

† Our attention was drawn to this possibility, which we had not considered, by Sir JOSEPH HOOKER in a letter to one of us. The passage containing the suggestion raises a point of the greatest interest and we quote it in full: "What can be the meaning of the capitate glands? They would seem to indicate the contemporaneous insect life which I think has been demonstrated to exist in the Coal Measures. Has any

is modified to form a pollen-chamber by the separation of its epidermis from the central tissue. Pollen-grains are found occurring in the bell-shaped chink thus formed.

The testa is coalescent with the body of the seed for four-fifths of its height. Around the apex of the nucellus it stands free as a complex chambered organ, the canopy. The micropyle does not extend as a pore or passage above the pollen-chamber, as in most other seeds, but encircles the narrow angled tube of the pollen-chamber like a spanner on a nut.

The free part of the integument or canopy is septate towards the apex, whilst lower down, the septa, usually nine in number, give place to deep furrows on the inner face. These furrows gradually flatten out as the plane of insertion of the canopy on the body of the seed is approached.

The outer surface of the testa is covered with a continuous palisade layer, the cells of which bear little peg-like outgrowths. At the summit the surface is thrown into sharp radially running ridges, nine in number, overlying the septa of the canopy.

The chalaza is of solid construction, and is penetrated by a single centrally placed vascular bundle which breaks up into nine peripheral strands. These run just below the surface of the seed and make their way one to each loculus of the canopy.

The seed was detached from its pedicel by a layer of abscission formed across the seed-base.

In addition to the full-sized seeds, smaller specimens occur. These are about $2\frac{1}{2}$ millims. in length and are generally found completely enclosed in the cupule, a husk-like sheath which springs from the pedicel just below the insertion of the seed. These small seeds have a full-sized pollen-chamber but contain no pollen. The testa is mature looking, and these seeds were probably abortive.

One specimen of an ovular stage has been found.

THE CUPULE.

The specimens at present known, in which the seed *Lagenostoma Lomaxi* is seen in connection with its cupule, are six in number. They are as follows :—

WILLIAMSON Collection, 1931	Longitudinal section.
" " 1931, A	" "
" " 1915, M	} The series of three transverse sections
" " 1915, O	
SCOTT Collection, 236	from the same seed.

one accounted for the quantity of pollen-grains in the sac of the ovule of Cycadeæ—so many more than the wind is likely to have brought?"

The suggestion, in any case a valuable one, gains in weight when it is remembered that the first account of the structure of a Coal-measure seed, in a paper that has become classic, was written by Sir JOSEPH HOOKER ("*Trigonocarpon*," 'Phil. Trans.' vol. 145, 1855), and that his conjecture that the flowers of *Welwitschia* were pollinated by insects has received general acceptance ('Linn. Soc. Trans.' vol. 24, 1863).

WILLIAMSON Collection, 1915, P.	Longitudinal, crushed.
SCOTT Collection, 558	„ showing pedicel.
U. C. L. Collection, R. 13	„ of young seed or ovule.

The most important sections showing the cupule detached from the seed are the following :—

R. Holloway College Collection, H. 55	Transverse section.
U. C. L. Collection, R. 14, α	Longitudinal and transverse sections, perhaps all from the same cupule.
„ „ R. 15	Longitudinal, showing pedicel.

Isolated fragments of the cupule are frequent, and in some cases, no doubt, belong to the same specimens which, in adjacent sections, are shown attached to the seed.

In the case of the detached cupules, the agreement in structure with those attached to the seed indicates *L. Lomaxi* as the species, but there is a bare possibility that some of these may represent the cupule (otherwise unknown) of *L. ovoides*, as the two species occur together.

From these various specimens a fair idea of the form and structure of the cupule and of its relation to the seed can be gained.

Form of the Cupule.

In some of the specimens showing seed and cupule in connection, the seed was of full size or nearly so* (the transverse series, W. 1915, M., &c., S. 558, and probably W. 1915, P.). In others the seed, though perfectly developed, is small (W. 1931 and 1931, A.), measuring 2.4 millims. and 2.2 millims. in length, as against 5 millims. or more in the mature seed. As pointed out above (pp. 195 and 211) these two specimens appear to represent an arrested condition of the young seed. In another case above described (p. 212) the seed, though scarcely smaller than the arrested specimens, appears to have been in the normal young or ovular condition, and is consequently badly preserved (U. C. L., R. 13). These three young or arrested specimens give the best idea of the general form of the cupule and its relation to the seed (see Plate 4, photos. 1 and 4 and Plate 10, fig. 34). At this stage the cupule far overtopped the seed, and no doubt completely enclosed it. In the youngest specimen (fig. 34) a lobe of the cupule even extends to double the height of the ovule, but here some displacement may have occurred. At maturity the enclosure of the seed was no doubt less complete. The transverse section W. 1915, M. (Plate 7, fig. 1) shows that even in the mature condition the cupule still reached to near the apex, but at this stage it

* Measurements of the seed are given above, p. 198.

was in all probability open at the top, as shown by the fact that most of the seeds found had freed themselves from it altogether.

The seed was borne terminally on a pedicel, as seen in the longitudinal section S. 558 (Plate 5, phot. 15 and Plate 10, fig. 33), the only case in which the connection is shown in a mature specimen; the seed, though cut tangentially, measures 4·5 millims. in length by 2·8 millims. in maximum width. The pedicel is shown for a length of about 2 millims., but as it is incomplete below, no idea of its real length can be formed. It is about ·5 millim. in diameter. The cupule springs from the pedicel at its upper end, immediately below the base of the seed, which it closely invests. In cases where the investment is loose this may be due to accident or to the arrested growth of the seed (Plate 4, phot. 1, from W. 1931, A.). The cupule in its lower



TEXT-FIG. 2.—Photograph from a large Wax Model of a Restoration of a small-sized Seed of *Lagenostoma Lomaxi*, enclosed in its Cupule. Model prepared by Mr. H. E. H. SMEDLEY.

part formed to all appearance a continuous envelope, as is shown most clearly in a section kindly lent by Miss BENSON (see Plate 7, fig. 7). This is a transverse section of a cupule, at once identified by the glands as belonging to the *Lagenostoma Lomaxi* type. The diameter is 3 millims. The seed itself is not present, but the central mass of tissue, which is partly preserved, agrees with that shown at the base of the seed in other specimens (e.g., S. 558, U. C. L., R. 15, cf. figs. 9 and 33, also photos. 15, 16). The dimensions also correspond, and there is little doubt that this section is from the lowest part of the cupule, on a level with the base of the seed. Here the cupule is evidently continuous all round, except for accidental fractures. We have no certain evidence to show to what distance upwards the cupule remained undivided. In the lowest of the series of three transverse sections (S. 236, see fig. 3) the cupular tissue is continuous for an extent of about 4 millims., representing about two-fifths of the periphery in this region. The rest of the cupule is much broken,

and one side of it bent back, the reflexed portion extending to the lobed region above (fig. 3). We have no evidence that the cupule divided into two valves, as in M. RENAULT's *Gnetopsis*;^{*} this is not impossible, but more probably it remained continuous until it broke up into the terminal lobes to be described below.

Returning to Miss BENSON's section, the cupule at this level is very conspicuously ribbed (fig. 7), a character less evident in the other specimen (*cf.* fig. 3). It appears even more deeply furrowed on the inner than the outer surface; there is no indication of this in other transverse sections, and it must probably be regarded as an effect of contraction and rupture of the tissues. The external ribbing was no doubt to some extent natural. Additional evidence for this is afforded by the longitudinal section S. 558 (Plate 5, phot. 15, and Plate 10, fig. 33), where the cupule appears about twice as thick on the left hand side as on the right. Presumably the section passed through a ridge on one side and a furrow on the other. A similar difference between the two sides is shown in the young specimen U. C. L., R. 13 (fig. 34). The number of ridges, if we may judge from the one complete transverse section (fig. 7), was about ten or twelve. It is difficult to determine whether all the existing protrusions were present during life.

In its upper part the cupule broke up into separate lobes. This is clearly shown in the small seed (W. 1931, A.) represented in longitudinal section in Plate 4, phot. 1. The section as a whole is median, but the part of the cupule to the right is evidently displaced, so as to be cut almost tangentially, thus showing two lobes connected below. Lower down on the same side a third lobe is seen. It is often difficult to make out to what extent the lobes were separate in the natural state. That laciniae existed is certain, as shown very clearly in the youngest specimen (U. C. L., R. 13, fig. 34), where a narrow lobe, bearing hairs on both sides, can be traced to its termination. It is probable, however, that the depth of the fissures between the segments may have been exaggerated by tearing or decay in the weak parts between the ridges, for the edges of the apparent lobes are by no means always clean and definite. In the transverse series of sections, which should throw most light on the segmentation of the cupule, this part of the specimen is unfortunately too fragmentary to give any certain information on the point. In the middle section (W. 1915, O., fig. 2) the portion of cupule at *c'* appears to have natural limits; this piece is about 2 millims. long, corresponding to about one-sixth of the periphery, it may represent two lobes not yet separated from each other. In the uppermost section of the series (W. 1915, M., Plate 7, fig. 1) there is still a continuous piece of cupule nearly as long as that in the previous sections, indicating perhaps that even so high up as this the lobes had not yet completely separated; the fragment in question is thinner at some points than at others, which may be a sign that further segmentation was in progress.

The specimen U. C. L., R. 14 (Plate 6, phot. 17, and Plate 8, fig. 11) shows the cupule

^{*} 'Cours de Bot. Foss.,' vol. 4, p. 180, Plate 20, figs. 7-10, 1885.

in approximately tangential section, parallel to the surface. It gives little definite evidence of lobing up to a height of about 3 millims. from the base; we cannot assume that its ragged edges really represent the outlines of natural segments; they may well be due to the section passing out somewhat obliquely through the ribbed surface.

From the data available we may conclude that the cupule of *Lagenostoma Lomaxi*, springing as a cup-shaped envelope from the pedicel, remained undivided for half its length or more, and then broke up into a number of lobes or laciniae; the latter may have become more deeply severed by subsequent splitting, which quite possibly occurred in the normal course of development.

Structure of the Cupule and Pedicel.

The cupule was traversed throughout its length by a number of vascular bundles, which branched off from the single strand of the pedicel.

The specimen which best exhibits the structure of the pedicel is the longitudinal section S. 558, already mentioned (Plate 5, phot. 15, and Plate 10, fig. 33). The section is tangential, as shown by its missing the micropyle and chalazal bundle of the seed; it approaches the median plane more nearly in its lower than in its upper part. The central bundle of the pedicel can be followed to within .5 millim. of the abscission-layer at the base of the seed; towards its upper end the bundle is clearly sending off a branch to the right, to enter the cupule; on the left a vascular bundle can be traced for a long distance through the cupule (phot. 16 and fig. 33), but its connection with the strand of the pedicel is missed.

The connection between the cupular strands and that of the pedicel is better shown in the young specimen, U. C. L., R. 13, which is seen in median section (fig. 34). Here the central strand can be followed all the way to the chalaza of the ovule, where the vascular system of the integument branches off (see above, p. 213). About .8 millim. below this point, a bundle diverges from the main strand at an acute angle, to gradually pass out into the cupule, through which it can be traced for some distance. Thus the relation of the bundle-systems of integument and cupule is clearly shown.

Another section of great value as showing the course of the cupular bundles is that represented in Plate 6, phot. 17, and Plate 8, fig. 11 (U. C. L., R. 14). This is the tangential section of the cupule; the base is towards the left of the figure. A number of vascular bundles (lettered *a-i* in the figure), are present; the principal strand (*a*), is about 300μ in diameter; it is only shown for a short distance and then branches out immediately into the three strands (*b*, *c* and *d*). The remaining bundles (*e-i*), also converge towards the principal strand or group, though their connection with it is missed. Probably all the bundles shown belong to the cupule, except the principal strand *a*, which, from its large size (much exceeding anything met with in the cupule

proper), can only represent the upper end of the central strand of the pedicel. The bundle *c*, which from its position, lying in the direct continuation of *a*, might be taken for the chalazal strand, is more probably one of the cupular bundles, for it differs in no way from the others, and no trace of the chalaza is to be found. The main strand *a* is obliquely cut, and its prolongation into the chalaza probably lay outside the plane of section. This specimen shows clearly how the main strand of the pedicel sent out branches on all sides into the cupule, which they traversed, eventually entering its terminal lobes (*cf.* fig. 34, from U. C. L., R. 13). That the cupular bundles themselves branched is certain, for the strands *g* and *h* (fig. 11), are clearly diverging from a common origin; a branching cupular bundle is also shown in phot. 1 (from W. 1931, A.), towards the right.

Our best evidence as to the number of bundles which entered the cupule is derived from the section (H. 55), lent by Miss BENSON; this, as we have seen, was cut transversely, about at the level of the base of the seed (Plate 7, fig. 7). The cupule here contains nine or perhaps ten recognisable bundles, of which two appear to be double and may represent points of branching. It is very likely that one or two other strands, no longer to be detected in the imperfectly preserved tissues, may have been present.

In the lowest section (S. 236), of the transverse series of three, cut at the level of the chalaza, seven bundles are shown in the very imperfect cupule (fig. 3). One of these strands is double and has evidently just branched. In the middle section of the same series, W. 1915, O., cut about half-way up the seed, seven bundles are seen in portions of the cupule occupying collectively scarcely half of the periphery (fig. 2); one of these strands is double.

It thus appears that the base of the cupule received about ten bundles (some of which may be branches of a smaller number, starting from the pedicel), and that these were increased, higher up in the cupule, by further branching.

We have now to consider the evidence as to the structure of the bundles, and will begin with that of the pedicel. Perhaps the most important section from this point of view is that illustrated in Plate 8, figs. 9 and 10 (U. C. L., R. 15). This section, as a whole, appears to be a tangential one through the pedicel and part of the cupule. Two of the characteristic glands are seen, one of which is *in situ*, leaving no doubt as to the identification with *Lagenostoma Lomaxi* or some closely similar form. The seed is absent, but appears to have been originally attached to the tissue marked *a*, as shown by the vertically elongated cells at this point, which exactly agree with those at the base of the seed in other specimens (*cf.* photos. 5 and 16, and fig. 33 from W. 1930 and S. 558). The part lying directly below the seed-base would thus be the pedicel; on the right, one side of the cupule is seen in position; the detached piece above may either be the left-hand side of the cupule displaced, or the distal part of the right-hand side, but the point is of little importance. The resemblance of the whole to the pedicel and cupule, as shown in S. 558 (phot. 16 and fig. 33), supposing the seed detached, is striking. Two bundles passing out to the cupule are

seen at the top of the pedicel (fig. 9, *v.b.*'), one in transverse, the other in longitudinal section; other strands traverse the cupule itself.

The pedicel, measured from the seed-base to its lower termination, is about 1.5 millims. in length, but is probably incomplete. The most important feature is the presence of a large vascular bundle, seen in somewhat oblique transverse section, near the lower end of the pedicel. This bundle, shown in detail in fig. 10, has a roughly triangular or three-lobed section; the maximum diameter of the xylem is about 300μ . The tracheides are cut obliquely, showing scalariform, or at some places pitted walls; they are commonly about 25μ in diameter, but some are much smaller, down to about 12μ . At three or four points there are gaps in the interior of the xylem, where the tissue has partly perished. These gaps were probably filled by parenchyma; in some there seem to be remains of disorganised tracheides. The smaller tracheides of the bundle border on the gaps, some or all of which probably represent the position of the protoxylem. That the latter was internal appears certain, for the peripheral elements are generally scalariform tracheides, of comparatively large size, and with no signs of disorganisation. The structure thus indicates that the bundle was a mesarch one; it was also, to all appearance, concentric, though the phloem region is too badly preserved to show any details. The bundle resembles that in a small rachis of *Lyginodendron Oldhamium* more nearly than any other form of vascular strand met with in these petrifications (*cf.* fig. 10 with Plate 6, phot. 27, from the rachis of *Lyginodendron*).

There can be little doubt that the bundle just described was that of the pedicel, which must have been bent at a considerable angle, so as to be cut somewhat transversely below, while seen in nearly longitudinal section in its upper part. The bundle corresponds with the large strand (α), seen in longitudinal section in U. C. L., R. 14, α (fig. 11), both in dimensions (diameter about 300μ in each), and structure. The tracheides are similar, and in the longitudinal section parenchyma occurs in the xylem, answering to the gaps in the less well-preserved transverse section. The bundle of the pedicel in S. 558 also agrees in structure, but its dimensions are much less, the xylem being about 150μ in diameter. It is probable, however, that this section is not median; the apparent diameter of the strand agrees very nearly with that of one of the projecting lobes of the xylem in U. C. L., R. 15.*

The anatomy of the pedicel is of considerable importance, as indicating that the organ on which the cupule and seed were borne was of a foliar nature. The structure is that of a petiole or rachis and not of a stem or branch.

* Another somewhat fragmentary longitudinal section of the pedicel occurs in section, U. C. L., R. 14, *b*. This may belong to the cupule of which portions are shown in the same slide and in the adjacent section, U. C. L., R. 14, α . It was clearly desirable to obtain a truly transverse section of the pedicel, and a search was made for the section next below the transverse series of three, often referred to above. Several sections from the same series were found; the only one which appears to come below the seed is in the Manchester Museum Collection, K₂. 14259. Several fragments of cupular tissue of *L. Lomaxi* are present, one of which contains a bundle not unlike that in U. C. L., R. 14, α , but smaller (175μ maximum diameter). It could not be referred with certainty to the pedicel.

The structure of the bundles in the cupule is fairly shown in several sections. Fig. 15 represents a transverse section of a bundle in a detached piece of cupule (shown in Plate 8, fig. 14) in U. C. L., R. 14, A., probably a fragment of the specimen shown in tangential section in the same slide (fig. 11). The structure of the bundle is to all appearance collateral; the small-celled tissue which alone can represent the phloëm is limited to one side of the strand; on the opposite side the parenchyma abuts immediately on the wood. The same collateral structure is shown in other preparations, e.g., W. 1915, M. and O. (fig. 16), from the series of three.

In the section represented in fig. 15, the orientation is inverted, the phloëm facing almost directly inwards. In most other cases, especially in the transverse series of three, the indications point to normal orientation (see fig. 16). The orientation may have really varied, perhaps in relation to the branching of the bundles, or the exceptional cases may be due to some displacement.

The evidence, though far from clear, owing to imperfect preservation, goes to show that the smaller cupular bundles at any rate were collateral, and in the majority of cases observed had normal orientation. In one or two cases the larger bundles of the cupule appear to have retained concentric structure.

The transverse section in fig. 15 does not clearly show the position of the protoxylem. In other sections, however (e.g., fig. 16, from W. 1915, O.), the smallest elements are almost always found near the middle of the xylem; in the finer strands they sometimes approach one or the other surface, but the mesarch arrangement is the rule. The longitudinal sections sometimes clearly show narrow spiral elements in the interior of the xylem (fig. 12) confirming the position of the protoxylem, as indicated in transverse sections. The tracheides are for the most part spiral or scalariform (fig. 12). Here and there, however, especially in the larger strands, elements with multiseriate bordered pits occur (see fig. 13 from the bundle *b* shown in fig. 11). These pitted elements exactly resemble those characteristic of *Lyginodendron Oldhamium*, and it is significant that in the foliar organs of that plant, just as in the cupule of *Lagenostoma Lomaxi*, spiral and scalariform tracheides preponderate, while the pitted elements, so general in the stem, are comparatively rare. In the finer strands of the lamina in *Lyginodendron* the pitted tracheides are wanting, and this seems to hold good for the finer cupular bundles of our *Lagenostoma*.

The vascular bundles of the cupule are enclosed in a conspicuous sheath, of large, rather thin-walled cells (see figs. 12 and 15) as much as 50μ in radial diameter. In this respect, again, they agree with the bundles in the leaflets of *Lyginodendron*, where the large-celled sheath is likewise a striking feature.* So far as comparison is possible the bundles in the cupule of *Lagenostoma* thus agree in all respects with those in the leaflets of *Lyginodendron*, while, as we have seen, the main strand of the pedicel in the former closely resembles that of the vegetative rachis in the latter.

As regards the parenchyma of the cupule there is little to be said; its preservation

* WILLIAMSON and SCOTT, "*Lyginodendron* and *Heterangium*," loc. cit., p. 731; Plate 24, fig. 16.

is always bad, even in the youngest specimens (see fig. 34, U. C. L., R. 13); the tissue between the bundles is reduced to a brown disorganised mass, in which mere traces of the cellular structure are recognisable. The peripheral zone is somewhat better preserved, and appears to consist of two or three layers of elongated elements, with walls rather thicker than those of the subjacent tissue. This darker zone sometimes contrasts rather sharply with the light brown of the disorganised parenchyma within (see figs. 1, 2 and 9). Elements with black contents, recalling the "secretory sacs" of *Lyginodendron*, can sometimes be detected in the cupular tissue, but their characters are not definite enough to help materially in identification. The most interesting point about the ground-tissue of the cupule is the fact that it was infested with fungi. Fungus-spores are almost always to be detected; in some specimens (e.g., U. C. L., R. 15) the tissues are completely filled with them, and they occur in such masses that they might be taken for a part of the normal structure, if it were not for comparison with other specimens in which the same bodies occur isolated. Fungal hyphæ are also found, sometimes forming dense strands following the course of the vascular bundles. All indications point to the cupule having already been an effete organ at the stage when its preservation took place, its condition contrasts markedly with that of the well-preserved adjacent fragments of the vegetative organs of *Lyginodendron* and other plants. This fact has an important bearing on the condition of the cupular glands, now to be described.

The glands form the most conspicuous feature of the cupule, by means of which even small fragments can usually be recognised at once; they occur on all parts of the cupule and pedicel (photos. 1, 4 and 15, figs. 1-3, 7 and 11) and are very numerous. Thus in the transverse section represented in fig. 7 no less than twenty-three are shown *in situ*.

The glands are massive structures, seated with a broad base on the cupule; sometimes they are practically sessile, but usually there is a distinct stalk, varying much in length (see photos. 1, 4, 16, 21 and 22, figs. 7, 14 and 17). In all cases the free end of the gland is formed by a large, more or less spherical head. In dimensions the head of the gland ranges from about 160-260 μ in diameter. The length of the gland as a whole varies much; the longest observed was 1.2 millims. in length (fig. 17), while the stalk was 600 μ thick at the base, diminishing to 120 μ at the narrowest part, just below the head, which was here 220 μ in diameter. As a rule the stalk is short—not much exceeding the diameter of the head—and sometimes there is no recognisable stalk at all, the spherical head being seated directly on the surface of the cupule (fig. 7).

The head of the cupular gland is in all cases a hollow structure; the shell of tissue enclosing the cavity is one cell thick at the free end, increasing to two cells or more at the sides, as the stalk is approached (see fig. 17 and phot. 21). The cavity is, as a rule, quite empty; at most only slight remains of disorganised tissue are found within it. The clearest case is that shown in phot. 23, where two or three fairly

definite cell-like bodies are seen inside the head ; they are probably not cells, but may represent the remnant of a broken-down tissue. Occasionally there are slight indications (from differences in colour) of the presence of some structureless organic substance within the gland. The cavity, as a rule, is approximately spherical, but sometimes it has an obovate or pear-shaped contour (see phot. 22) ; this may depend on the less complete disappearance of the internal tissue.

The stalk, when present, is a solid column of tissue many cells thick. It is never found well preserved, but a peripheral zone of elongated, apparently thick-walled cells, continuous with the outer layer of the cupule, can sometimes be distinguished (phot. 21 and fig. 17). The form of the internal cells of the stalk is obscure, owing to the state of preservation, but seems to have varied with that of the gland as a whole, the cells being more elongated in the longer stalks. Where the gland is sessile, the wall of the head gradually thickens towards the base, and passes over directly into the adjacent tissue of the cupule.

No trace of a vascular supply has been found in connection with any of the glands.

In a few cases the tissue of the head or stalk has been thrown into spiny elevations, no doubt by unequal contraction of the inner and outer layers.

The structure of the cupular glands will be further considered in the next section, where they are compared with those occurring on the vegetative organs of *Lyginodendron*.

Not uncommonly, multicellular hairs occur on the cupule ; several are present, for example, on the specimen shown in transverse section in fig. 7. They are most conspicuous, however, on the cupule of the young seed or ovule (U. C. L., R. 13) represented in fig. 34, where they occur in abundance on a long lobe of the cupule, forming a conspicuous tuft at its extremity (*h*). On the older cupules they are but sparsely present ; probably they belonged to the young condition of the organ, and were shed as it advanced in age. The hairs are cylindrical structures (fig. 8), consisting of a single row of three or four cells, or perhaps more, for they are rarely seen complete. The cells are about 20μ in diameter, and range in length from about $30-60\mu$.

Summary of Cupule.

The chief facts with regard to the cupule of *Lagenostoma Lomaxi* may be briefly summed up as follows :—

The young seed was enclosed in a cupule or envelope exceeding the seed in length. Mature seeds are sometimes found still within the cupule, but are more often free.

The cupule was borne terminally on a pedicel ; it formed a continuous ribbed cup below, and divided above into a number of lobes or segments.

The pedicel contained a single vascular bundle, which, before entering the chalaza, gave off numerous branches into the cupule, traversing it throughout its length and extending into the segments.

The bundle of the pedicel appears to have had a concentric mesarch structure, while its branches in the cupule became collateral, the orientation, in the majority of cases, being normal.

Large and prominent multicellular glands of capitate form were borne on the cupule and pedicel; the head of the gland, in the usual state of preservation, is hollow.

Uniseriate hairs were also borne on the cupule, and are chiefly found on the younger specimens.

COMPARISON WITH THE VEGETATIVE ORGANS OF *Lyginodendron*.

The first and most important point to be considered is the comparison of the glands, for it was the resemblance between the glands occurring on the cupule of *Lagenostoma Lomaxi* and those already known on the vegetative parts of *Lyginodendron* which first suggested a connection between these fossils.

The characteristic cortical emergences, which form a constant feature of the stem and leaves of *Lyginodendron Oldhamium*, have been familiar since WILLIAMSON first described them in 1890.* Glandular outgrowths, however, do not seem to have been distinguished till a few years later, in 1895.† They are by no means equally frequent in all specimens; in some of the material they may long be sought in vain, while in other cases they are extremely abundant, and are met with in practically every section of the stem or leaf. The form of *Lyginodendron* occurring at Dulesgate, with which the specimens of *Lagenostoma Lomaxi* are closely associated, is exceptionally rich in glands. They are present alike on stem, petiole and rachis, and on the leaflets themselves. These vegetative glands are massive capitate structures, usually with a stalk of varying length, but occasionally sessile.

The largest glands generally occur on the stem. The gland shown in Plate 8, fig. 18, borne on the cortex of a stem, evidently of a considerable size, may serve as a fair example of the long-stalked form. Here the whole length is 2.2 millims.; the stalk is about 700 μ thick at the base, and about 200 μ in the upper part, the head having a diameter of about 300 μ . On other stems, glands as much as 3 millims. in length have been observed, but most of the stem-glands are much shorter. The longer glands seem to have easily become detached, for isolated specimens, exactly agreeing with those found elsewhere in connection with the stem, are common in the Dulesgate material, where they occur mixed up with the seeds of *Lagenostoma Lomaxi*. On the petiole and rachis the long type of gland is comparatively rare, though here also a length of as much as 2 millims. is sometimes reached. Usually the petiolar glands

* "Organisation of Fossil Plants of Coal-measures, Part XVII," 'Phil. Trans.,' B, vol. 181 (1890), p. 90, figs. 1, 6, 7, 8, and 9.

† WILLIAMSON and SCOTT, "Further Observations on the Organisation of Fossil Plants, &c., Part III.," 'Phil. Trans.,' B, vol. 186 (1895), p. 730, Plate 23, fig. 12.

are short—about $300\text{--}400\mu$ in length—with heads varying from about $160\text{--}240\mu$ in diameter; sometimes the glands are sessile, and this is commonly the case where they are found on the pinnules of the leaf.

We have seen above (p. 223) that the cupular glands of *Lagenostoma Lomaxi* may attain a length of 1.2 millim., but are more commonly short, ranging usually from a total length of about 500μ down to the sessile form, while the diameter of the head varies from about $160\text{--}260\mu$. Thus the dimensions of the cupular glands are practically identical with those of the vegetative glands, the agreement being most exact with the glands that occur on the petiole and rachis of the leaf. The form is also the same, and shows about the same range of variation (compare, for example, phot. 16 from a cupule with phot. 19 from a petiole).

The structure of the vegetative glands is well exhibited in many specimens, as shown in Plate 6, photos. 24–26, and in Plate 8, fig. 18. Their preservation is generally far more perfect than that of the cupular glands. A particularly good example is represented in phot. 24, from a detached gland, occurring in the same block from Dulesgate which yielded most of the specimens of the seed. It is a long gland (1.2 millims. in total length) with the head of the maximum dimensions, about 400μ in diameter; it was probably borne on the stem, as it agrees closely with glands found *in situ* on stems of *Lyginodendron* from the same locality.

The interior of the head is occupied by a mass of small cells, which we regard as constituting the secretory tissue of the organ. The general shape of the secretory mass is obovate; the cells are for the most part perfect, but some larger lumina in the lower part of the group, may indicate that here a lysisigenetic process was beginning. At the free end the boundary wall of the head is formed by a single layer of narrow cells; lower down the wall increases in thickness, especially on one side, as regards both the number and width of its cells. The stalk is bounded throughout by a well-marked epidermis of somewhat elongated cells; the depression near the base (*st.* in phot. 24) no doubt indicates the position of a stoma. The internal tissue of the stalk consists in the upper part of elongated cells, while towards the base the elements are shorter; the thickness of the cell walls diminishes from without inwards. Dark coloured cells scattered in the internal tissues resemble the “secretory sacs” of the stem and petiole.

Essentially the same structure is shown in the long gland from the stem, represented in fig. 18, and, generally, all the vegetative glands in question exhibit the same features,* apart from insignificant variations. Where the gland as a whole is short the cells of the stalk are usually short (also see phot. 20). In no case was any sign of a vascular strand observed in connection with a gland.

Allowing for the inferior preservation of the cupule of *Lagenostoma Lomaxi* as compared with that of the vegetative organs of *Lyginodendron*, it is evident that the

* On some stems much smaller and simpler gland-like bodies occur side by side with those described. They do not affect the present question and will be dealt with on another occasion.

glands of the one agree very closely with those of the other. In dimensions and form, and in the structure of the stalk, there is in fact no distinction to be drawn between them. As regards most of the specimens, however, there is this difference: in all cupular glands observed the head is empty, or very nearly so, while in the great majority of vegetative glands it is filled with a small-celled tissue, usually well-preserved. So long as this difference proved constant it was impossible to regard the two organs as identical. On searching, however, through large numbers of sections of *Lyginodendron*, cases were soon met with in which the vegetative glands were in the same condition as those on the cupule of the *Lagenostoma*. The first instance of this was observed in slides 1915 B. and 1415 C. of the WILLIAMSON Collection, cut from the same block from which most of the specimens of the seed were derived. A small rachis, about 2 millims. in maximum diameter, with the typical *Lyginodendron* structure, appears in each of these sections; it bears glands of the usual vegetative type, but with the head completely hollow, just as in the cupular glands.* In the two sections three of these hollow glands are shown. The clearest of the three has a length of 320μ , with the head 190μ in diameter. Subsequently, an even better instance of the hollow state of preservation of vegetative glands was found. The specimen is a petiole or rachis of *Lyginodendron*, measuring about 4×2 millims. in transverse section. One section is in D. H. SCOTT'S Collection (S. 625) and four others are in the collection at University College (U. C. L., M. 11, a-d).

It was from this specimen that the illustrations (photos. 18, 19 and 20) were taken. A large number of glands are present, and in all those in which the head is shown, of which there are eleven in the three sections particularly studied, its cavity is perfectly empty. The glands vary in size; that shown in phot. 20 is among the largest, it measures 400μ in total length and the head is about 240μ in diameter. The agreement with the cupular glands is strikingly exact, as shown, for example, on comparing phot. 20, from the petiole, with phot. 21, from the cupule; both photographs are on the same scale. In some of these vegetative glands (phot. 20) the tissue of the stalk is completely preserved, as is usually the case in the cupular glands.

We thus find that the vegetative glands of *Lyginodendron*, when in a certain condition of preservation,† agree in all points with those on the cupule of *Lagenostoma Lomaxi*. It is probable that the hollow state of the head in these glands is due to the breaking down of secretory tissue during life, rather than to *post-mortem* decay. Intermediate conditions are sometimes found in the vegetative glands. In the specimen shown in phot. 25, for example, the secretory tissue is beginning to show signs of disorganisation, and where this is the case there is some resemblance to the fragments of broken-down tissue seen in the cupular gland represented in phot. 23. In several vegetative glands (see phot. 26) a definite gap had formed in the middle of the secretory mass. We thus see that there is a series of cases connecting the

* The authors are indebted to Miss M. C. STOPES for the detection of this valuable piece of evidence.

† Additional examples of this state of preservation have since come under observation. June 25, 1904.

hollow gland characteristic of the cupule, with the fully preserved condition which is more usual on the vegetative organs.

The fact that the cupular glands are almost always found with the head empty is no doubt to be explained by the effete condition of the cupule at the time it was preserved, as indicated by the state of all its tissues. Its functional activity must have been limited to a stage earlier than any which has as yet come under observation, though in its old age it may still have served as a protective husk. So far as the glands are concerned, the agreement between the cupule of *Lagenostoma Lomaxi* and the vegetative organs of *Lyginodendron Oldhamium* is thus found to be complete. No other fossil plant is known which bears glands at all comparable to those described.

As regards the vascular system, the main points of comparison have already been indicated and may be shortly recapitulated here. The vascular bundle of the pedicel in *Lagenostoma Lomaxi* agrees in form, and in its mesarch and to all appearance concentric structure, with the bundle of a small petiole or rachis in *Lyginodendron Oldhamium* (see Plate 8, fig. 10 and compare Plate 6, phot. 27).

The bundles of the cupule of *Lagenostoma* in usually assuming collateral structure with mesarch xylem, agree with those in the pinnules of *Lyginodendron*. They further correspond in possessing a conspicuous, large-celled bundle-sheath. In the smaller strands of the cupule only spiral and scalariform elements are found, while in the main strand of the pedicel and the bundles immediately springing from it, elements with multiseriate bordered pits also occur. In these respects, again, there is an exact agreement with the bundles of the leaflets and rachis respectively of *Lyginodendron*. The evidence from the vascular structure thus completely confirms the attribution first suggested by a comparison of the glands.

The preservation of the other tissues of the cupule is not sufficiently good to admit of any profitable comparison with the vegetative organs. The moribund condition of the cupule, as indicated by the constant presence of fungal spores and hyphæ, sufficiently accounts for its inferior state of preservation as compared with that of the foliage leaves of *Lyginodendron*.

The comparison between the structure of *Lagenostoma Lomaxi* and that of *Lyginodendron* has been based entirely on the cupule and pedicel of the former. The seed itself is so highly specialised an organ that no detailed agreement with vegetative structures is to be looked for. At the same time, there is certainly no discrepancy. It may be mentioned in this connection that in the closely allied *Lagenostoma ovoides* "secretory sacs," resembling those of *Lyginodendron*, have been observed by one of us in the parenchyma filling the loculi of the canopy.

It must be remembered that the argument from detailed correspondence of structure is strengthened by that from intimate association. Evidence of the latter kind, though worthless by itself, is by no means without weight as an auxiliary to the former. The specimens of *Lagenostoma Lomaxi* everywhere occur mixed up with the

stems, leaves, and roots of *Lyginodendron Oldhamium*, and it is with the peculiarly glandular form of the "species" that the seeds, with their glandular cupules, are associated.* Where vegetative and reproductive organs presenting identical structural features, not known to occur in other plants, are thus found in close and constant association, the inference that the one belonged to the other appears irresistible. Evidence from actual continuity is always the final goal in questions of attribution among fossil plants; the seed of *Lagenostoma Lomaxi* has not yet been found *in situ* on the *Lyginodendron* plant, but short of this the evidence for the one belonging to the other could scarcely be stronger than it is.

THE POSITION OF THE SEED ON THE PLANT.

Until the seed has been found in actual connection with the plant to which it belonged, we are necessarily left without any direct evidence as to how the fructification was borne. As already pointed out, the anatomical evidence clearly indicates that the cupule together with the pedicel was of a foliar nature. This foliar organ may represent either an entire sporophyll, borne with its fellows on some axial structure, or a modified pinnule of a compound leaf, corresponding to the sterile foliage of the plant. Either interpretation is possible.

Thanks to Mr. LOMAX's observations we now know that the stem of *Lyginodendron* was branched;† in some forms of the plant the ramification was many times repeated, and it is possible that some of the ultimate branches may have assumed the character of floral axes, though we have as yet no evidence that this was the case. The peculiar "bud-like structure" described in 1895‡ might be thought of in this connection. A number of specimens of the same nature have come to light since this description was published, and a series of sections recently received from Mr. LOMAX shows conclusively that the "bud-like structure," in some cases at all events, was nothing but a very young stem or branch, densely clothed with long and often glandular emergences. There is also evidence that similar young or arrested branches occasionally occurred in the axils of the leaves on the largest stems. At present, however, there is nothing to show that any of these branches were connected with reproduction.

It is unlikely in any case that a definite strobilus was formed; the radial symmetry of the seed and cupule and the absence of any signs of mutual compression indicate that the fructification—whatever its morphological nature—was a lax one.

The alternative hypothesis, that the seeds were borne on the frond, is at least

* In one case a leaflet of *Lyginodendron* is found enclosed within the cupule of a seed. See phot. 4 (W. 1931).

† J. LOMAX, "Some New Features in Relation to *Lyginodendron Oldhamium*," 'Annals of Botany,' vol. 16, December, 1902.

‡ WILLIAMSON and SCOTT, *loc. cit.*, p. 732, Plate 24, figs. 14 and 15.

equally probable, though for this also we have no direct evidence; no structures suggesting fertile fronds or fertile pinnæ have as yet been detected in the petrified specimens of *Lyginodendron*. The very abundant and often well-preserved foliage appears to have been all of the familiar sterile type. Nor, so far as we are aware, has *Sphenopteris Höninghausi*, which is identical with the foliage of *Lyginodendron* preserved in the form of impressions, been found as yet in any but the sterile form. STUR, however, has attributed certain fertile pinnæ to the closely similar species *Sphenopteris Stangeri*, of lower carboniferous age, and probably this attribution is correct, though evidence from actual continuity is wanting. The fructification borne on the fertile pinnæ is *Calymmatotheca Stangeri*.* If *Calymmatotheca Stangeri* belonged to a *Lyginodendron*, as appears probable, it may possibly represent the male fructification of the plant (see below, p. 238). It would, even in that case, however, establish a certain presumption that the female reproductive bodies might have been borne in a similar way on the frond.

Mr. KIRSTON's important discovery of the seeds of *Neuropteris heterophylla*,† announced during the writing of the present paper, shows that in this member of Cycadofilices the seed was borne on a pinna of the frond. The position is apparently terminal, but may have been lateral originally. The analogy of this plant, though not belonging immediately to the same family as *Lyginodendron*, no doubt somewhat increases the probability that the seeds were borne on the frond in the latter genus. A further analogy may perhaps be found in a fossil about to be described by Mr. ARBER in conjunction with one of the present writers, where there is reason to suspect that the numerous *Lagenostoma*-like seeds may have belonged to the rachis associated with them, possibly a fertile frond of the *Sphenopteris* type. Other specimens, showing seeds *in situ*, point in the same direction.

It is possible that among recent Ferns the ancient and now monotypic genus *Thyrsopteris* may give us the best idea how the fructification of *Lyginodendron* was borne. Here the branched fertile pinnæ, springing from the main rachis of the frond, terminate in the sori, each surrounded by its cupule-like indusium; in the Palæozoic plant similar branched pinnæ may have borne sometimes the cupulate seeds, sometimes the pollen-bearing sporangia. On this view the pedicel of our *Lagenostoma* would represent an ultimate ramification of the fertile rachis.

MORPHOLOGY.

In its whole organisation *Lagenostoma Lomaxi* recalls so vividly the seed of a modern Cycad that it is unnecessary here to do more than recapitulate, in the briefest way, the characters which mark it out for inclusion in the class of structures termed seeds.

* STUR, "Die Culmflora," 'Abhandl. K. K. Geol. Reichsanstalt' (Vienna), vol. 8, 1877.

† "On the Fructification of *Neuropteris heterophylla*, BRONGNIET," read before the Royal Society on December 3, 1903 ('Phil. Trans.' B, vol. 197, p. 1).

Lagenostoma has an integument with micropyle, and there is a permanently enclosed megaspore containing a prothallium. Pollination no doubt took place on the parent plant, and the structure was then detached as a whole through the agency of the layer of separation.

In the extrusion of mucilage from its integument *Lagenostoma Lomaxi* recalls an adaptation widely prevalent among the seeds of higher plants; an adaptation which though trifling in itself, helps to confirm the belief that the integument had already become an integral portion of the seed.

The main point in which *Lagenostoma* diverges from the typical seed is in the lack of an embryo, a negative character common to the whole series of fossil Gymnospermous seeds that have been recovered from the Palæozoic rocks. This point is discussed elsewhere in the present paper and need not be pursued here. These earlier seed-types perhaps retained the Fern-like mode of continuous development from the fertilised egg-cell to the exclusion of any definite resting period such as we usually associate with a "ripe seed." If any pause occurred it may perhaps have immediately preceded fertilisation and have coincided with the maturing of the microspores in the pollen-chamber and the liberation of spermatozoids. This interpretation, too, seems consistent with the processes as we know them in living Pteridophytes.

The seed of *Lyginodendron* differed from recent seeds in the early maturing of its tissues. Already before fertilisation has taken place they appear to have reached the limits of their development, and would be incapable of further stretching. The ovular stage, *i.e.*, the persistence of an embryonic condition of the tissues of nucellus and integument till after fertilisation had occurred, was a later adaptation.

There is another feature in which our seed is peculiar amongst the known seeds of this period. The micropyle is the pore of access to the nucellus, and in other seeds the microspores must needs traverse it on the way to effect a lodgment in the pollen-chamber. Here, this is unnecessary, seeing that the micropyle is disposed as a collar around a pollen-chamber whose orifice communicates directly with the outside.* The integument covers the seed, leaving exposed the spot appropriated to the reception of the pollen. It is possible we have in this feature a reminiscence of the pollination of an ancestral naked megasporangium.

We may pass on now to a consideration of the homologies of the several parts of the seed and of the enclosing cupule. The modified apex of the nucellus, the pollen-chamber, is even more highly specialised than the corresponding structure among the Cycads. For the bell-shaped, crevice-like form of the cavity in *Lagenostoma* connotes a relatively high degree of precision in the movements of the spermatozoids, and affords ground for the suspicion that the distribution of the archegonia will be found

* A similar relation obtains in the genus *Arucaria*, cf. STRASBURGER, 'Die Coniferen und die Gnetaceen,' p. 65 and Plate 7, fig. 56. The same appears to hold good for *Sequoia*; LAWSON, Gametophytes, &c., of *Sequoia sempervirens*, 'Ann. of Bot.,' vol. 18, January, 1904, p. 6.

to have a similar relation. Efficiency in the primitive types of seeds seems to have depended on the perfection of the pollen-chamber. Later came an advance from the side of the microspore; the pollen-tube was evolved, and upon its appearance followed the decline of the pollen-chamber.*

Under the circumstances it is hardly to be expected that the pollen-chamber of *Lagenostoma* should offer any evident indication of the manner of its origination from a Fern sporangium.

The peculiar integument of the seed demands some consideration. Distally it consists of the chambered canopy which freely surrounds the apex of the nucellus, whilst proximally it is coalescent with the lower four-fifths of the seed. It was a vascular structure, supplied with bundles from the vascular strand that entered at the chalaza. These bundles, as they run in the wall of the seed, lie well within the integumental region, as defined by continuing backwards the actual plane of separation of nucellus and integument. There can be no question in this case of a nucellar origin of the bundles.

The second point to be noted is that of the possible multiple character of the integument in its free part, as suggested by the chambering of the canopy. In *Lagenostoma Lomaxi* the several loculi of the canopy are as completely fused with one another as are the carpels in a syncarpous gynæceum, though, of course, near its insertion the lining of the canopy is evidently grooved, and even where the grooves disappear, giving place to radial septa, indications of the double origin of the septa are still discernible for some distance. Taken in conjunction with WILLIAMSON'S *Lagenostoma physoides*,† a seed still under re-investigation, in which the canopy seems to be represented by a whorl of free tentacles inserted below and enfolding the pollen-chamber, a multiple origin for the integument would appear not improbable.

The outer envelope of the seed, the cupule, is, of course, a much less specialised organ than the integument, and, as we have shown, has much in common with the ordinary vegetative pinnules of *Lyginodendron*.

Translated into appropriate Filicinean terminology, we have in *Lagenostoma* a megasporangium which has been enclosed in two successive, concentric, indusium-like structures, of which the inner has become an integral portion of a new organ, the seed. The outer is probably of later origin, and would appear to have afforded protection to the seed only when the latter was quite young. It is quite possible that the two enclosures have originated very similarly, i.e., as peltate, lobed structures, and that the present integument was once a comparatively unspecialised cupule-like indusium. An exact parallel is hardly to be found among recent Ferns, though a certain analogy is offered by the case of *Onoclea sensibilis*, in which each sorus is enclosed by its own proper indusium as well as by an infolded marginal lobe of the pinnule. *Azolla*, too, may be cited in this connection. In this genus the sori—each

* Cf. 'Annals of Botany,' vol. 17, p. 452.

† WILLIAMSON, "On the Organisation, &c., Part VIII.," 'Phil. Trans.,' vol. 167, fig. 77.

with its own close-fitting indusium—are inserted in pairs upon the ventral lobe of the sporophyll, while from the base of the dorsal lobe a general sheath, or “involucre,” is provided, which forms an enclosure for the group of sori.*

In the present state of our knowledge the term *indusium* carries no very precise morphological signification, and conflicting views have been expressed as to its origin and nature. In such groups of the Polypodiaceæ, however, as Dicksoniæ and Davalliæ, the ontogenetic indications are consistent with a marginal or terminal origin for the sorus, whilst the indusium has arisen from more or less connate marginal outgrowths, which form a theca around the sorus. The relations in *Lagenostoma* appear to justify a comparison with the groups cited, whilst the Hymenophyllaceæ offer a further parallel. That the enclosures of *Lagenostoma* should retain their vascular strands, whilst the indusia in most Ferns are non-vascular, is no bar to the existence of a homology between these structures. In the great majority of cases a flimsy, reduced structure is best adapted to the discharge of its functions by a Fern indusium, though instances occur in which the indusium is penetrated by vascular bundles.†

In *Lagenostoma*, on the other hand, special reasons may exist for the retention of the bundles. Reference has already been made to a possible function of these strands in the canopy (see p. 214); whilst in the cupule, which is richly vascular, there is, of course, the secretory function of the numerous glands.

The cupule may be regarded as a comparatively new structure, dating its origin, perhaps, from the time when the seed habit was acquired, and the old or primary indusium (the canopy) became incorporated as an integral portion of the seed.

We have heard with much interest and appreciation of the views of Miss M. BENSON upon the homologies of this seed.‡ Following PRANTL,§ this botanist regards the seed as the equivalent of a sorus, but, unlike PRANTL, she interprets the integument (the canopy of *Lagenostoma*) as a reduced or sterilised series of sporangia. The seed, as a whole, is regarded as a metamorphosed synangium, of which the central sporangium alone retains its pristine function.||

We turn now to a consideration of the possible relations of the more primitive Gymnospermous seeds to *Lagenostoma*.

First, we would cite *Gnetopsis elliptica*,¶ from the French Permo-carboniferous of Grand' Croix. This is the only known fossil seed possessing a cupule, though in this

* GOEBEL, 'Organographie,' pp. 669, 670.

† PRANTL, 'Unters. zur Morphologie d. Gefässkryptogamen,' Heft 1, "Die Hymenophyllaceen," pp. 36, 37, and fig. 97.

‡ M. BENSON, 'Ann. of Bot.,' vol. 18, p. 161, January, 1904.

§ PRANTL, 'Unters. zur Morphologie d. Gefässkryptogamen,' Heft 2, 'Die Schizaceen,' p. 153.

|| It would unduly prolong the present paper to enter here on any discussion of this important suggestion, which has been published since our memoir was communicated to the Royal Society.

¶ Cf. RENAULT, 'Cours de Bot. Fossile,' vol. 4, p. 180, Plate 20, 1885.

case the relations differ from *Lagenostoma* in that the cupule contained as many as four seeds. This seed, like our own, shows indications of a complicated integument, which suggests comparison with the canopy. The pollen-chamber, also, seems to have possessed a central cone not unlike the one we have described. Whatever may have been the plant that bore this seed, the analogies which the latter presents with *Lagenostoma* are most striking.

Amongst existing Gymnosperms, the Taxacæ at once recall the *Lagenostoma* condition in the frequent possession of a second integument (aril). In *Taxus* and *Phyllocladus* the aril is still free from the seed; in *Torreya* it is adnate throughout the greater portion of the seed, whilst in *Cephalotaxus* it may possibly be represented by the outer fleshy covering of the testa.

At present it is undesirable to press these comparisons too closely. The suggestions made are tentative and provisional, and may need revision, as further additions to our knowledge of the mode of fructification of the Cycadofilices accrue.

A comparison of the seeds of Cycads with *Lagenostoma* is inevitable. The vegetative organs of *Lyginodendron* possess marked Cycadean features, whilst the seed, which we attribute to this fossil, has an essentially Cycadean organisation. The canopy of a *Lagenostoma* may well have undergone simplification into the hard integument of a Cycadean seed, and, in that case, the vascular strands, which run in or near the plane of union of nucellus and integument in the latter, should correspond with the integumental bundles of *Lagenostoma*, even though they no longer pass into the free part of the integument.

Whether the fleshy sarcotesta of *Cycas*, with its vascular strands, correspond to a completely adnate cupule, may, perhaps, be left an open question. In any case, the detailed structure of Cycadean seeds is a subject fully deserving further attention and elucidation.

THE SYSTEMATIC POSITION OF *Lyginodendron*.

When BINNEY, in 1866, first described his newly-discovered *Dadoxylon Oldhamium*, no doubt of the Gymnospermous affinities of the plant seems to have occurred to the mind of its discoverer. "It evidently belonged," he says, "to the genus *Pinites* of WITHER, since changed by ENDLICHER and BRONGNIART into *Dadoxylon*."* WILLIAMSON'S full investigation of the structure of the stem, published in 1873,† left the taxonomic relations of the plant doubtful, but removed it definitely from the *Dadoxylon* lumber-room; WILLIAMSON had, in fact, already separated it in 1869, under the generic name *Dictyoxydon*.‡ Speaking of *Lyginodendron Oldhamium*,

* BINNEY, "On Fossil Wood in Calcareous Nodules found in the Upper Foot Coal, near Oldham," 'Lit. and Phil. Soc. Proc.,' Manchester, vol. 56, p. 113, read February 20, 1866. BINNEY, however, compared the primary wood of his stem to that of *Sigillaria elegans*.

† "Organisation of Fossil Plants of Coal-measures, Part IV.," 'Phil. Trans.,' 1873.

‡ "On the Structure and Affinities of some Exogenous Stems from the Coal-measures," 'Monthly Microscopical Journal,' vol. 2, p. 66, 1869.

as he named BINNEY's plant in 1873 for the first time, and of the allied *Heterangium Grievii*, WILLIAMSON says: "The question of the botanical position of both the above plants remains undecided, and I confess I shrink from arriving at a decision in the present state of our knowledge respecting them. If I succeed in establishing a connection between the common Oldham plant and the equally common *Edraxylon* [*Rachiopteris aspera*, of later memoirs], then, strange as it may appear, the former will become an undoubted arborescent Fern; at the same time, it has many features of affinity with the Lycopodiaceæ that must not be overlooked."* He goes on to speak of the difficulty of distinguishing between the fossil stems of Lycopods and Ferns, but adds, in a footnote, that more recent researches had rendered it increasingly probable that *Heterangium Grievii* was a true Fern. He always recognised the close affinity between the two genera, an opinion which more recent work has all tended to confirm.

It is remarkable that WILLIAMSON at that time seems only to have hesitated between Lycopods and Ferns, and to have lost sight for the moment of the indications of Gymnospermous affinity which these plants present. Perhaps this was a natural reaction against BINNEY's too confident reference of the Oldham plant to *Dadoxylon*.

In his memoir of 1874,† WILLIAMSON described *Rachiopteris aspera*, as he now called the petiole previously named *Edraxylon*, and compared it with certain Fern-fronds known as impressions, *Sphenopteris Hönninghausi*, BRONGNIART, among others. But he says nothing more here of any relation between this petiole and the *Lyginodendron*-stem.

In 1887‡ WILLIAMSON returned to this question, and expressed his conviction that the petiole and stem belonged to the same plant; the conclusion "that *Lyginodendron Oldhamium* belongs to the group of Ferns" has, he says, "now made a near approach to certainty" (*loc. cit.*, p. 298); he extends the same view to *Heterangium*. A few lines further on he says, of these two genera, that "possibly they are the generalised ancestors of both Ferns and Cycads," citing *Stangeria* as a living plant retaining a similar combination of characters (*loc. cit.*, p. 299). In this passage WILLIAMSON clearly expresses the idea of an intermediate group, but with him the balance still inclined to the Fern side, for in 1890,§ after showing by identity of structural characters (the cortical emergences in particular) "that *Rachiopteris aspera* is merely a petiole of *Lyginodendron Oldhamium*," he adds: "it also follows that *L. Oldhamium* is a true Fern, most probably belonging to some Sphenopterid type" (*loc. cit.*, p. 91). In his General Index, too (1894), *Lyginodendron* is included under Filices.||

* *Loc. cit.*, p. 405.

† "Organisation, &c., Part VI.," 'Phil. Trans.,' B, vol. 164, Part II.

‡ *Loc. cit.*, Part XIII., 'Phil. Trans.,' B, vol. 178 (1887).

§ *Loc. cit.*, Part XVII., 'Phil. Trans.,' B, vol. 181 (1890).

|| WILLIAMSON, "General, Morphological, and Histological Index, &c.," 'Manchester Lit. and Phil. Soc. Mem. and Proc.,' 1893-4.

In the meantime FELIX, who found *Lyginodendron Oldhamium* in the Coal-measures of Westphalia, had suggested in 1885 a comparison with Cycads, and more especially with Medulloseæ, which were then classed with them.* In 1887, COUNT SOLMS-LAUBACH in his Introduction to Fossil Botany,† recognised with special reference to *Lyginodendron*, the existence of groups intermediate between Ferns and Gymnosperms. In subsequent memoirs he goes further, and strongly emphasises the significance of extinct types (*Lyginodendreae* among others), combining the characters of Filicineæ and Gymnosperms, and indicating the common origin of the two classes.‡

In the memoir on *Lyginodendron* and *Heterangium*, published in 1896 by Professor WILLIAMSON, in conjunction with one of the present writers,§ it was pointed out that the available evidence clearly indicated a position intermediate between Ferns and Cycads (*loc. cit.*, p. 704), and that the two genera are best regarded as the derivatives of an ancient and generalised Fern-stock, from which they already show a marked divergence in the Cycadean direction. In the absence of evidence as to the fructification the question whether it was more likely to have been Filicinean or Gymnospermous in character was regarded as perfectly open.

In 1897 POTONIÉ, in his textbook,|| established the class Cycadofilices for the group of Palæozoic plants, including *Lyginodendron*, already recognised as combining Cycad-like with Fern-like characters. His proposal has been generally adopted by subsequent writers on the subject.

In the same year ZEILLER¶ first called attention to the probability that the fructification of *Lyginodendron* was of the type of STUR's *Calymmatotheca Stangeri*; on the ground of this attribution ZEILLER inclined to the view that *Lyginodendron* was a true Fern, and was disposed to assign the same position to the other genera in question. In his 'Éléments de Paléobotanique' (1900), while recognising the group Cycadofilicineæ as distinct and without any direct analogue in the recent flora, he says that it would be rash to exclude them, on their vegetative characters alone, from the Ferns (*loc. cit.*, p. 125); as regards *Lyginodendron* in particular his opinion is that, judging from its probable *Calymmatotheca* fructification, it appears

* "Strukturzeigende Pflanzenreste a. d. oberen Steinkohlenformation Westphalens," 'Ber. d. Naturforsch. Gesellschaft z. Leipzig,' 1885, p. 7.

† English edition, pp. 141, 163, and 362.

‡ See, for example, his memoirs on *Protophytes Buchiana*, 'Bot. Zeitung,' 1893, p. 207, and on *Medullosa Leuckartii*, 'Bot. Zeitung,' 1897, p. 198.

§ WILLIAMSON and SCOTT, "Further Observations, &c., Part III.," 'Phil. Trans.,' B, vol. 186 (1895).

|| 'Lehrbuch der Pflanzenpaläontologie, &c.,' 2, Lieferung, 1897, p. 160. POTONIÉ has changed the name *Lyginodendron* to *Lyginopteris*, on the ground that the former genus was originally founded by GOURIAE on Lepidodendroid casts. This alteration has been adopted by some other palæobotanists; for reasons of convenience we have preferred to retain WILLIAMSON's nomenclature.

¶ "Sur quelques Fougères des Dépôts houillers d'Asie Mineure," 'Soc. Bot. de France Bull.,' vol. 44 (1897) p. 199.

to have been a true Fern.* Up to that time no one had expressed a doubt that *Calymmatotheca*, the only fructification which then appeared referable to *Lyginodendron*, was Filicinean. Hence, in 1900, one of the present writers, in a general survey of the Cycadofilices,† based the intermediate position of *Lyginodendron* and its allies wholly on evidence drawn from the structural characters of the vegetative organs, pointing out "that if we were to rely on reproductive characters alone, we should probably, on the existing evidence, agree with M. ZEILLER in regarding *Lyginodendron* as still belonging to the Filicales."‡

The position, then, previous to the present investigation was this: the evidence for the existence in Palæozoic times of an extensive group of plants transitional between Ferns and Cycads appeared overwhelmingly strong, and was regarded by certain botanists, including the present writers, as justifying the conclusion that the Cycadales, and not improbably the Gymnosperms generally, were derived from a Filicinean ancestry.§ This conclusion then depended entirely on a detailed comparative study of the vegetative, and especially of the anatomical characters. The evidence as to the reproductive organs of the Cycadofilices was extremely scanty: in *Lyginodendron*, where alone any significant data were available, there was a presumption that the fructification was of a type (*Calymmatotheca*) then generally accepted as Filicinean.

The results of the present investigation have placed the whole question in a new light. If our evidence be accepted—and short of the proof of the continuity it could scarcely be stronger—it follows that *Lyginodendron*, so far as the female fructification is concerned, had definitely crossed the boundary between Cryptogams and Spermatophytes; as regards its seeds it was as true a Gymnosperm as any known Palæozoic plant. The morphology of the seed is discussed in another section (p. 230); here we need only say that though some features in its organisation may fairly be interpreted as primitive, the seed nature is as little open to doubt as in any Spermatophyte of Palæozoic age. Thus the centre of gravity of the group (for, as is already clear, other Cycadofilices will prove to be likewise involved) has undergone a marked displacement in the Gymnospermous direction. Those who view intermediate groups with suspicion will now have to class *Lyginodendron* not as a true Fern, but as a true Gymnosperm, and that, indeed, would be its correct position if we were justified in simply applying the scheme of recent taxonomy to Palæozoic plants. *Lyginodendron* conforms, as nearly as any known contemporary plant, to the characters on which the division Gymnospermæ is based. Yet the features on which an intermediate position between Ferns and Cycads was originally assigned to the

* *Loc. cit.*, p. 129. See also the author's discussion of the whole question, pp. 369–371.

† SCOTT, 'Studies in Fossil Botany,' 1900, Lectures X. and XI. Here the anatomical evidence is fully stated, and references will be found to the original memoirs dealing with the various plants in question.

‡ *Loc. cit.*, p. 336.

§ SCOTT, *loc. cit.*, pp. 514–522.

genus, retain all their former significance. In the whole of the foliar characters, whether structural or external, in the primary structure of both stem and root, and in the relation between the stem and the roots, *Lyginodendron* shows unmistakable affinity with the Ferns, and certainly stands incomparably nearer the Cryptogams of that class than does any plant included among recent Gymnosperms.

The question as to the nature of the male fructification still remains open. STUR's reference of his *Calymmatotheca Stangeri* to a *Sphenopteris*, almost identical with the foliage of *Lyginodendron Oldhamium*, is probably correct, but it is by no means certain that the *Calymmatotheca* in question represents a microsporangiate fructification. An examination of the original specimens, kindly lent for that purpose by the Director of the Geol. Reichsanstalt at Vienna to the Geological Department of the British Museum, leads us to accept STUR's interpretation of the tufted bodies terminating the pinnules as valves of a foliaceous "indusium," rather than to regard them with M. ZEILLER as of a sporangial nature.* Whether the cupule-like indusium enclosed a seed or a microsporangial sorus has not yet been determined; the actual reproductive organs, whatever they may have been, appear to have been shed previous to fossilisation.

MISS BENSON has discovered a fructification, with structure preserved, which she places in a new genus, *Telangium*, and is inclined to regard as the pollen-bearing synangium of *Lyginodendron Oldhamium*, with which the specimens occur in association.† If this attribution should prove to be correct, it would follow that the microsporangia of *Lyginodendron* had much in common with the fructification of undoubted Ferns, and the evidence for the Filicoid affinities of the genus would thus be further strengthened. For the present, however, it is safest to leave the microsporangia out of account, as this question is still involved in so much doubt.

On the whole of the evidence actually before us, the position of *Lyginodendron* as a member of a group of plants, transitional between Filicales and Gymnosperms, appears to be definitely established. While many of the Filicoid characters are retained, the plant, in the organisation of its seed, had fully attained the level of a Palæozoic Gymnosperm.

The absence of any indication of an embryo within the ripe seed is a negative character which our plant shares with all the known Spermatophytes of its period. Strictly speaking we have no proof that any of these plants possessed seeds corresponding to the current definition, in which the presence of an embryo forms an essential character.‡ On this ground it might appear desirable to establish a group

* The statement of this question given on a former occasion by one of us (SCOTT, 'Studies in Fossil Botany,' p. 325) now requires modification in the sense indicated above.

† 'The Fructification of *Lyginodendron Oldhamium*,' 'Annals of Botany,' vol. 16, 1902. Full paper since published, 'Ann. of Bot.,' vol. 18, p. 161, 1904.

‡ See above, p. 231; also SCOTT, "Origin of Seed-bearing Plants," lecture delivered before the Royal Institution, May 15, 1903, p. 6.

analogous to, though by no means co-extensive with the Progymnosperms of SAFFORD and MARION,* for the Palæozoic Spermatophytes. Such a group would embrace all the plants of that period of which the seeds are known, including the Cordaitæ. The latter, however, approach much nearer to the Gymnosperms than is the case with *Lyginodendron*, as shown by their whole vegetative organisation, by their complex inflorescence, and generally, by their wide divergence from the Fern type, from which they appear far more remote, even than recent Cycads. For these reasons we do not propose to disturb the Cordaitæ in the place among Gymnosperms which has been generally accorded them. To do so would involve a re-consideration of the position of the recent Cycadaceæ and of *Ginkgo* in which fertilisation also takes place at a late stage, while the degree of development of the embryo within the ripe seed appears to be very variable. It is, however, desirable that some group, more definite than the Cycadofilices, should be formed for those Palæozoic plants, of Filicinean affinities, which prove to have possessed seeds. Mr. KIDSTON's discovery of the seed of *Neuropteris heterophylla*, already referred to (p. 230) indicates that such a group will soon be well furnished with members. There is abundant evidence that the *Neuropteris* fronds were the foliage of some of the Medulloseæ; there is thus every reason to anticipate that the whole of this family, so well characterised anatomically and morphologically, will prove to have been seed bearing; there is already some direct evidence for the attribution of the seed *Trigonocarpon olivæforme* to a *Medullosa*,† and these indications are much strengthened by the analogy of Mr. KIDSTON's *Neuropteris*. Thus it appears that the Medulloseæ as well as the Lyginodendreæ are rapidly qualifying for a place among primitive Spermatophyta.

The present group or association Cycadofilices was founded expressly on vegetative and anatomical characters,‡ it has served its purpose in emphasising the existence of a great plexus of Palæozoic plants combining Filicinean with Gymnospermous features. Now, however, we are in a position to advance further, for we know that some at least of these transitional types, while retaining much of the Filicinean organisation, had already entered the ranks of seed-bearing plants. It appears to us that the presence in the Palæozoic flora of these primitive, Fern-like Spermatophytes, so important as a phase in the history of evolution, may best be recognised by the foundation of a distinct class which may suitably be named *Pteridospermeæ*. The proposed class may be provisionally defined as embracing those Palæozoic plants with the habit and much of the internal organisation of Ferns, which were reproduced by means of seeds. A more exact definition must await more extended knowledge of the reproductive characters; it is, however, not improbable that the radial symmetry of the seed, and the occurrence of fertilisation at a late stage, when the ovule had already acquired the mature structure of a ripe seed, as well as the postponement

* 'L'Évolution du Règne Végétal.—Les Phanérogames,' vol. 1, pp. 17, 62, and 68.

† G. WILD, "On *Trigonocarpon olivæforme*," 'Manchester Geol. Soc. Trans.,' vol. 26, 1900.

‡ See POTONIÉ, 'Lehrbuch,' p. 160.

of embryonic development, may prove to have characterised these early seed plants.

We shall probably incur little risk of error if we already place the *Lyginodendreae* and *Medulloseae* in the new class *Pteridospermeae*. The group *Cycadofilices* will continue to be employed for those transitional families, where we have as yet no evidence to show how far the reproductive advance had proceeded. Such a family for example, as the *Cladoxyleae*, must remain for the present in a wholly undetermined position, for we have no indications here to show whether anatomical progress had or had not been accompanied by any marked advance on Cryptogamic methods of reproduction.

The further development of our knowledge of the *Pteridosperms* will form one of the chief objects of palæo-botanic investigation in the near future.

In conclusion the authors desire to express their thanks to Mr. J. LOMAX, whose skill and good judgment in the collection and preparation of the material have been of essential service; all the sections of the seed which WILLIAMSON appropriately named in his honour, are from his hand. They are further greatly indebted to Miss M. C. STOKES, for her invaluable assistance, at a critical stage of the investigation, in obtaining evidence by searching the WILLIAMSON and other extensive collections, and to Mr. L. A. BOODLE who, in addition to contributing a large part of the illustrations, has been in close touch with the investigation throughout.

EXPLANATION OF PLATES 4-10.

Plates 4-6.—Photographs from the sections. In most cases they should be examined with the aid of a hand-lens. Photographs 1-7 and 14-27 are by Mr. L. A. BOODLE. Photographs 8-13 by Mr. W. TAMS, of Cambridge.

PLATE 4.

Phot. 1.—Longitudinal section of a small arrested seed enclosed in its cupule, which loosely invests it on all sides. The cupule bears numerous glands with hollow heads. In the seed the testa, chalaza, canopy, pollen-chamber, and other parts are seen, as shown more in detail in photos. 2 and 3.

W. 1931, A. × about 15. (See pp. 211 and 216.)

Phot. 2.—Upper part of the same seed. *t*, testa, consisting of young palisade layer and hypoderm; *l*, a loculus of the canopy; *s*, sinus, or space between inner wall of canopy and wall of pollen-chamber; *p. c*, the bell-shaped pollen-chamber surrounding the central cone, which is not quite in median section; *p. c'*, tube of chamber, projecting beyond the micropyle.

W. 1931, A. × about 36. (See p. 200.)

Phot. 3.—Lower part of the same seed, slightly overlapping the part shown in phot. 2. *a. l*, abscission-layer; *ch. c*, the bulky chalazal cushion, displaced upwards, as seen in phot. 1; *v. b*, vascular bundle of chalaza, branching out above into bundles which traverse the contracted intermediate sac, *i. s*; *mg.*, membrane of megaspore, which is seen complete, but contracted.

W. 1931, A. × about 45. (See p. 212.)

Phot. 4.—Another small cupulate seed, in tangential section. *c.*, cupule, bearing many glands; *gl.*, the gland represented in phot. 22. Above the seed the cupule encloses a pinnule of *Lyginodendron*, *Ly.*; *a. l.*, abscission-layer; *ch.*, chalaza; *t.*, testa, with conspicuous hypoderm; *s.*, sinus; *w.*, wall of pollen-chamber in tangential section; *m.*, micropyle. In the body of the seed three vascular bundles are seen.

W. 1931. × about 20. (See pp. 211, 212.)

Phot. 5.—Full-sized seed in longitudinal section. At the chalaza the section is fairly median, but at the micropylar end it misses the central cone of the pollen-chamber. Otherwise all the parts of the seed are shown, as represented in detail in photos. 6 and 7. *mg.*, contracted membrane of megaspore.

W. 1930. × about 10. (See p. 198.)

Phot. 6.—Upper part of the same seed. *pal.*, palisade layer of testa; *o. t.*, outer, *i. t.*, inner wall of canopy, the loculus between them contains some remains of tissue; *s.*, sinus; *w.*, wall of pollen-chamber, projecting slightly beyond micropyle at *p. c.*; *mg.*, membrane of megaspore, within which remains of prothallial tissue are seen.

W. 1930. × about 26. (See p. 200.)

Phot. 7.—Lower part of the same seed. *a. l.*, abscission-layer; *ch. c.*, chalazal cushion; *v. b.*, vascular bundle of chalaza (*cf.* Plate 7, fig. 5); *i. s.*, intermediate sack; *mg.*, membrane of megaspore contracted.

W. 1930. × about 26. (See p. 207.)

PLATE 5.

Phot. 8.—Slightly oblique longitudinal section of a full-sized seed. The pollen-chamber (*p. c.*) is cut in a tangential plane and shows two groups of pollen-grains (*p. g.*). *c. c.*, central cone of pollen-chamber; *o.*, orifice of pollen-chamber; *s.*, sinus round the pollen-chamber; *l.*, loculus of canopy; *t.*, testa. The lower end of the section falls outside the abscission-layer. The pollen-chamber of this specimen is enlarged in Plate 9, fig. 21.

U. C. L., R. 2. × about 16. (See p. 200.)

Phot. 9.—Longitudinal section of a full-sized seed. The section falls outside the pollen-chamber at the apex, but approaches the axis of the seed in its lower part. *se.*, *se'*, complete and incomplete septa of canopy; *l.*, loculus of canopy; *pl.*, plinth or mound upon the summit of which the pollen-chamber stands; *i. s.*, intermediate sack contracted; *v. b.*, *v. b.*, vascular bundles; *mg.*, contracted membrane of megaspore containing prothallial tissue; *nu.*, tissue at base of nucellus.

U. C. L., R. 3, *a.* × about 13. (See p. 203.)

Phot. 10.—Very oblique section across the top of a full-sized seed, cutting two of the loculi of the canopy tangentially in the plane of the vascular bundles. *p. c. w.*, wall of pollen-chamber; *c. c.*, central cone of pollen-chamber; *l.*, *l.*, loculi of canopy cut in tangential section; *v. b.*, *v. b.*, vascular bundles of these loculi, abutting below on the bundle-ring; *se.*, *se.*, septa separating the loculi; *pal.*, *hy.*, palisade and hypodermal layers of testa; *r.*, *r.*, ridges around the micropyle, cut obliquely.

U. C. L., R. 9, *c.* × about 40. (See p. 204.)

Phot. 11.—Testa from specimen in phot. 9 (right hand, at base), more highly magnified, and rotated through 90°. *pal.*, palisade-layer bearing pegs (*pe.*, *pe.*) with normal preservation; *hy.*, hypoderm of testa; *nu.*, tissue at base of nucellus; *ch. c.*, chalazal cushion.

U. C. L., R. 3, *a.* × about 46. (See p. 206.)

Phot. 12.—Transverse section of testa of full-sized seed, with palisade cells and mucilage cones. *pal.*, palisade cells; *mu.*, mucilage cones; *pe.*, position of peg raised on a mucilage cone; *hy.*, hypoderm of testa.

U. C. L., R. 8, *b.* × about 100. (See p. 206.)

Phot. 13.—Approximately transverse section of the chalaza of a full-sized seed with chalazal vascular bundle (testa shown on lower side of photograph only). *v. b.*, vascular bundle; *ch. c.*, chalazal cushion; *pal.*, palisade-layer of testa.

U. C. L., R. 8, *a.* × about 45. (See p. 208.)

Phot. 14.—Transverse section of one of the vascular bundles of the seed (that marked *v. v.* in Plate 7, fig. 2). *i. s.*, part of the "intermediate sack" on which the bundle abuts; *mg.*, part of the megaspore membrane. In the bundle only the xylem is evident; the smaller elements are on the whole directed towards the inner side.

W. 1915, O. × about 200. (See p. 210.)

Phot. 15.—Longitudinal section of cupulate seed and pedicel. The section is obliquely tangential, approaching the median plane at the lower end. *p.*, pedicel on which the seed is borne; *c.*, *c.*, cupule, bearing glands; on each side it extends a little above the reference-line; *t.*, testa of seed; *ch. c.*, chalazal cushion. Cf. Plate 10, fig. 33. Portions of the seed are shown enlarged in photos. 16 and 21.

S. 558. × 13. (See p. 217.)

PLATE 6.

Phot. 16.—Chalazal portion of the seed shown in phot. 15. *c.*, cupule, showing a vascular bundle and bearing several glands; that marked *gl.* is shown on a larger scale in phot. 21; *ch. c.*, chalazal cushion; *pal.*, palisade-layer; *hy.*, hypoderm of testa.

S. 558. × about 35. (See p. 217.)

Phot. 17.—Detached cupule, seen in approximately tangential section; the base of the cupule is towards the left; several glands are shown; that marked *gl.* is represented further enlarged in phot. 23. The course of the bundles traversing the cupule is shown in the semi-diagrammatic fig. 11, Plate 8.

U. C. L., R. 14, *a.* × 28. (See p. 219.)

Phot. 18.—Transverse section of a small petiole of *Lyginodendron Oldhamianum*, showing the characteristic vascular bundle and hypoderm. Numerous glands are present, in three of which the head is seen to be hollow; cf. phot. 19.

S. 625. × 15. (See p. 227.)

Phot. 19.—Portion of the same petiole enlarged, showing the three glands with the head in the hollow state of preservation. *hy.*, hypoderm, with radial sclerotic bands.

S. 625. × 36. (See p. 227.)

Phot. 20.—Gland from another section of the same petiole, for comparison with the gland shown in phot. 21 from the cupule of *Lagenostoma Lomaxi*. The head of the gland is completely empty, while the tissue of the stalk is well preserved. *hy.*, sclerotic band of hypoderm.

U. C. L., M. 11, c. × 70. (See p. 227.)

Phot. 21.—Gland (marked *gl.* in phot. 16) from the cupule of *Lagenostoma Lomaxi*, for comparison with the petiolar gland shown in phot. 20. *v. b.*, a vascular bundle of the cupule.

S. 558. × 70. (See pp. 223 and 227.)

Phot. 22.—Gland (marked *gl.* in phot. 4) on the cupule of a small seed. Note the pear-shaped cavity of the head. *c.*, cupule; *pal.*, palisade-layer; and *hy.*, hypoderm of the testa of the seed.

W. 1931. × 54. (See p. 224.)

Phot. 23.—Gland (marked *gl.* in phot. 17) from a cupule. *gl. t.*, remains of glandular tissue in the cavity of the head. Below, a part of the vascular bundle *d.* (see Plate 8, fig. 11) is seen. *sh.*, *sh.*, large-celled bundle-sheath.

U. C. L., R 14, a. × about 60. (See p. 223.)

Phot. 24.—Detached vegetative gland, to show the typical structure. Compare Plate 8, fig. 18, where a similar though longer gland is shown *in situ* on the stem. *gl. t.*, glandular tissue of the head, almost perfectly preserved. The structure of the stalk is also well shown. *st.*, depressed stoma.

S. 447. × about 60. (See p. 226.)

Phot. 25.—Detached vegetative gland. The glandular tissue, *gl. t.*, has here become somewhat disorganised.

W. 1931, A. × about 80. (See p. 227.)

Phot. 26.—Detached vegetative gland. Here the glandular tissue, *gl. t.*, is breaking down, showing a lenticular gap in the middle.

W. 1931, A. × about 80. (See p. 227.)

Phot. 27.—Vascular bundle, in transverse section, from a small rachis of *Lyginodendron Oldhamium*, to compare with the bundle from the pedicel of the seed shown in Plate 8, fig. 10. Only the xylem is preserved; *px.*, position of median protoxylem group. Lateral protoxylem was no doubt present also, to the right and left.

S. 448. × about 80. (See pp. 221 and 228.)

PLATES 7-10.

Figures from camera lucida drawings. Those in Plates 7 and 8 by Mr. L. A. BOODLE; Plate 10, figs. 27, 28, 28A and B, and 34 by Miss M. C. STOPES; all the other figures by one of the authors.

PLATE 7.

Figs. 1-3.—Series of three approximately transverse sections of the same seed; the orientation of the sections is uniform throughout the series.

Fig. 1.—Uppermost section, passing through the canopy. *c.*, *c.*, portions of the cupule, containing vascular bundles and bearing glands; *pal.*, palisade; *hy.*, hypoderm of testa, cut obliquely; *l.*, *l.*, loculi of the canopy (nine in all), separated by radial septa; *i. t.*, inner wall of canopy; *p. c. w.*, wall of pollen-chamber; *c. c.*, central cone of pollen-chamber.

W. 1915, M. × 18½. (See pp. 198, 204, and 218.)

Fig. 2.—Middle section through the body of the seed. *c*, *c'*, portions of the cupule, of which many fragments are shown; *c'*, the largest continuous piece (a number of glands and vascular bundles are shown); *v. b.*, the bundle represented in fig. 16; *pal.*, palisade; *hy.*, hypoderm of testa; *i. s.*, intermediate sack on which the nine vascular bundles abut; *v. b'*, the bundle represented in Plate 5, phot. 14; *mg.*, membrane of megaspore.

W. 1915, O. $\times 18\frac{1}{2}$. (See pp. 198 and 218.)

Fig. 3.—Lowest section, through chalaza. *c*, *c'*, cupule, which is here nearly complete; *pal.*, *hy.*, palisade and hypoderm of testa, cut obliquely; *ch. c.*, chalazal cushion; *v. b.*, vascular bundle of chalaza, shown enlarged in fig. 4.

S. 236. $\times 18\frac{1}{2}$. (See pp. 198 and 218.)

Fig. 4.—Vascular bundle of chalaza and surrounding tissues, transverse section. In the xylem the smallest elements are nearly central (*cf.* fig. 5). The phloëm is not well defined.

S. 236. $\times 90$. (See pp. 209 and 210.)

Fig. 5.—Vascular bundle and surrounding tissues of chalaza in longitudinal section. *px.*, central protoxylem of bundle; *nu.*, tissue at base of nucellus; *ch. c.*, part of the chalazal cushion (*cf.* Plate 4, phot. 7).

W. 1930. $\times 98$. (See pp. 209 and 210.)

Fig. 6.—Part of a vascular bundle from a full-grown seed, in approximately radial section. *i.*, inner; *o.*, outer side; *px.*, protoxylem element. The other tracheides are spiral or scalariform, and in one case pitted. Some of the narrow elements adjacent to the xylem may be phloëm.

W. 1931, A. $\times 390$. (See p. 210.)

Fig. 7.—Transverse section through the lower part of a cupule, bearing numerous glands and containing vascular bundles. *c*, *c'*, cupule; *b. s.*, *b. s.*, tissue at base of seed; *h.*, hair represented in fig. 8. The specimen is split into two unequal parts by a wide crack.

Section lent by Miss BENSON. H. 55. $\times 18\frac{1}{2}$. (See p. 217.)

PLATE 8.

Fig. 8.—Hair, from the cupule shown in fig. 7.

H. 55. $\times 390$. (See p. 224.)

Fig. 9.—Cupule and pedicel in approximately tangential section. *p.*, pedicel; *c*, *c'*, cupule, one portion of which is detached; *gl.*, gland *in situ*; *b. s.*, tissue at base of seed, which is lost; *v. b.*, vascular bundle of pedicel, represented enlarged in fig. 10; *v. b'*, bundles passing out into cupule.

U. C. L., R. 15. $\times 18$. (See p. 220.)

Fig. 10.—Vascular bundle of pedicel shown in fig. 9, in somewhat oblique transverse section. Some of the gaps in the wood probably represent position of protoxylem.

U. C. L., R. 15. $\times 175$. (See p. 220.)

Fig. 11.—Cupule, in approximately tangential section, shown also in Plate 6, phot. 17. *a*, vascular bundle of pedicel; *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, vascular bundles of cupule; *gl.*, *gl.*, glands.

U. C. L., R. 14, *a*. $\times 18$. (See p. 219.)

Fig. 12.—Part of the vascular bundle marked *a* in fig. 11, showing xylem with phloëm on each side. *px.*, protoxylem; *ph.*, *ph.*, phloëm; *sh.*, *sh.*, bundle-sheath.

U. C. L., R. 14, *a*. $\times 150$. (See p. 222.)

Fig. 13.—Part of the vascular bundle marked *b.* in fig. 11, showing a large tracheide with multiserial bordered pits.

U. C. L., R. 14, *a.* × 390. (See p. 222.)

Fig. 14.—Detached portion of cupule, bearing glands. Two vascular bundles are shown; that marked *c. b.* is represented in fig. 15. The arrow has the same direction as in that figure.

U. C. L., R. 14, *a.* × 18½. (See p. 222.)

Fig. 15.—The vascular bundle marked *r. b.* in fig. 14. *x.*, the large-celled xylem; *ph.*, phloem; *sh.*, bundle-sheath. The arrow, as in fig. 14, points outwards, showing that the orientation of the bundle is, in this case, reversed.

U. C. L., R. 14, *a.* × 390. (See p. 222.)

Fig. 16.—Another vascular bundle from a cupule (that marked *v. b.* in fig. 2). *px.*, protoxylem, in the interior of the xylem strand; *ph.*, phloem. The arrow points outwards (parallel to the axis of the adjacent gland, see fig. 2), and thus the orientation is here normal.

W. 1915, O. × 390. (See p. 222.)

Fig. 17.—Long gland borne on a cupule. *c.*, cupular tissue; *gl. t.*, remains of glandular tissue in cavity of head.

U. C. L., R. 14, *b.* × 38. (See p. 223.)

Fig. 18.—Long gland, borne on a stem of *Lyginodendron Oldhamium*. *co.*, cortex of stem; *gl. t.*, glandular tissue of the head, perfectly preserved. The peripheral wall of the head is broken at the top.

S. 1781. × 38. (See p. 225.)

PLATE 9.

Figs. 19–25, *Lagenostoma Lomaxi*.

Fig. 19.—Nearly median longitudinal section of the pollen-chamber and adjacent parts of a full-sized seed. *p. c.*, cavity of pollen-chamber; *p. g.*, pollen-grain (two are present); *c. c.*, central cone of pollen-chamber; *o.*, orifice of pollen-chamber; *s.*, sinus between canopy and pollen-chamber; *pl.*, plinth supporting the pollen-chamber; *i. t.*, inner wall of canopy or integument; *o. t.*, outer wall of canopy; *r.*, ridge on testa radiating from micropyle. The ridge is cut obliquely, owing to the section being slightly tangential and oblique; *mg.*, crumpled wall of megaspore.

U. C. L., R. 1. × 60. (See p. 200.)

Fig. 20.—Oblique section of a full-sized seed. The section starts near the micropyle, cutting the two middle loculi of the canopy as it enters almost transversally. It then strikes the pollen-chamber at mid-height, and, sloping down, cuts its base almost at the full diameter; continuing, the section cuts the intermediate sack and megaspore obliquely and travels out of the seed about two-thirds down the side remote from that at which it entered. *l.*, loculus of canopy; *s.*, septum of canopy (still showing the compressed groove in its median plane); *r.*, ridges on surface overlying the septa of the canopy; *p. c.*, pollen-chamber; *p. g.*, pollen-grain; *c. c.*, central cone of pollen-chamber; *s.*, sinus around plinth and pollen-chamber; *v. b.*, *v. b.*, vascular bundles abutting on the intermediate sack (*i. s.*); *mg.*, megaspore.

Section lent by Professor F. E. WEISS. Manchester Collection, K₂, 14257. × 27. (See p. 200.)

Fig. 21.—A somewhat tangential section of a pollen-chamber of a full-sized seed. *p. c. w.*, wall of pollen-chamber. Below, in the middle of the figure, just where the pollen-chamber is constricted, a portion of the wall is cut tangentially. *p. g., p. g.*, pollen-grains lying in the cavity of the pollen-chamber (about half a dozen are present); *c., c.*, central cone of tissue; *o.*, orifice of pollen-chamber; *r.*, ridge on outside of canopy, cut obliquely; *i. t.*, inner wall of canopy; *s.*, sinus round pollen-chamber; *pl.*, plinth.

U. C. L., R. 2. × 62. (See p. 200.)

Fig. 22.—Oblique section across the canopy and pollen-chamber of a full-sized seed. The section entered the seed at the very edge of the micropyle, where it cuts three of the ridges (*r. r.*) obliquely. It passes obliquely into the neck of the pollen-chamber (*p. c.*), in leaving which it follows the plane of the wall (*p. c. w.*) for some distance. Below and flanking the pollen-chamber is the gap or sinus (*s.*). Two loculi of the canopy (*l. l.*) are cut into on either side, and the lower pair of loculi show their vascular strands (*v. b., v. b.*) lying in the plane of section. Part of the inner tangential wall of a fifth loculus is seen as an island of tissue (*i. t.*); *se., se.*, septa separating loculi. The transverse bar below these structures is the bundle-ring (*b. r.*). It is continued up on the left side as far as the second septum. The section passes out of the seed a little more than one-third of way down.

KIDSTON Collection, 260. × 20. (See p. 200.)

Fig. 23.—Transverse section across the canopy and pollen-chamber of a small-sized seed. *p. r. w.*, wall of pollen-chamber, shifted somewhat from its true position. On the left the cells are much displaced; *s.*, sinus; *i. t.*, inner, fluted face of canopy; *f.*, groove on inner face of canopy; *l., l.*, loculi of canopy; *se., se.*, septa—both those shown are double; *v. b.*, vascular bundle lying in a loculus; *o. t.*, outer wall of canopy.

U. C. L. Collection, R. 11. × 95. (See p. 203.)

Fig. 24.—Median longitudinal section of a young abortive seed. The pollen-chamber (*p. c.*) stands in the centre of the figure with its central cone of tissue (*c., c.*) rising from the base. The tube of the pollen-chamber (*p. c.*) projects from the micropyle. Separated from the wall by the sinus (*s.*) is the canopy. *i. t.*, inner wall; *o. t.*, outer wall of canopy; *r.*, ridge at the micropyle. The plinth was undeveloped at this stage of development.

W. 1931, A., small seed. × 65. (See p. 200.)

Fig. 25.—An oblique section across the canopy of a full-sized seed. As in fig. 22, this section enters the seed close to the micropyle cutting across a pair of ridges (*r.*) very obliquely; it then (unlike fig. 22) slopes away from the pollen-chamber, cutting the middle loculus of the canopy (*l.*) parallel to its inner wall. *l., l.*, other loculi of canopy; *a.* and *b.*, vascular bundle cut twice, once below the canopy (*a.*) and again as it runs up the loculus (*b.*); *b. r.*, bundle-ring; *s.*, sinus; *s. e.*, septum of canopy; *i. t.*, inner wall of canopy. The section leaves the seed about half-way down the same side as that which it entered.

KIDSTON Collection, 424. × 21. (See p. 204.)

PLATE 10.

Figs. 26–34, *Lagenostoma Lomazi*.

Fig. 26.—Slightly oblique longitudinal section of the chalaza of a full-sized seed. *a. l.*, marks the position of the abscission-layer; *ch. c.*, the chalazal cushion; *pal.*, the palisade layer, thinning out at the seed-base; *hy.*, the fibrous hypoderm; *v. b.*, three of the branch vascular strands shown, one in transverse, the other two in somewhat oblique section. The main chalazal bundle does not lie in the plane of section; *nu.*, tissue at base of nucellus.

U. C. L. Collection, R. 4. × about 60. (See p. 207.)

Fig. 27.—Oblique section through the chalazal region of a full-sized seed. Starting rather near the periphery of the scar and passing upwards and inwards, the section strikes the axis of the seed just at the point of branching of the chalazal bundle. Here the bundle *a* is cut nearly transversely, *b* longitudinally, and *c* obliquely. Three bundles run entirely outside the plane of section, whilst the remaining three, *d*, *e*, and *f*, are cut higher in their course after they have turned the corner of the intermediate sack. *a*, *l*, abscission-layer; *ch*, *c*, fibres of the chalazal cushion, cut nearly transversely in the lower part of the figure; *nu*, tissue at base of nucellus; *pal*, palisade-layer.

Section lent by Professor C. E. BERTRAND, Lille, Lab. Bot. Collection, 1110. $\times 43$. (See p. 207.)

Figs. 28, 28A, and 28B.—Palisade cells with pegs from full-sized seed.

All from Lille, Bot. Lab. Collection, 1108.

Another section from the seed given in fig. 27.

$\times$ about 180. (See p. 206.)

Fig. 28.—A palisade cell (*pal*) with peg (*pe*).

Fig. 28A.—Two palisade cells with pegs. This figure shows the most usual type of preservation.

Fig. 28B.—Several palisade cells with pegs raised to various heights by mucilage cones. *pal*, palisade cells; *hy*, hypoderm; *pe*, *pe*, pegs; *mu*, mucilage-cone.

Fig. 29.—Portion of a transverse section of the wall of a full-sized seed from about the middle part of the seed showing a vascular bundle cut *in situ*. *pal*, palisade-layer; *pe*, peg; *hy*, hypoderm, with "thick" preservation; *v. b.*, vascular bundle; *mg*, megaspore.

U. C. L. Collection, R. 7, *b*. $\times$ about 100. (See p. 209.)

Fig. 30.—Portion of a transverse section of a full-sized seed cut at a height corresponding to the insertion of the pollen-chamber. *pal*, palisade-layer; *o. s.*, outer sack of the seed; *b. r.*, bundle-ring; *v. b.*, vascular bundle; *i. t.*, internal wall of canopy.

U. C. L. Collection, R. 6, *c*. $\times 32$. (See p. 203.)

Fig. 31.—Portion of intermediate sack (*i. s.*) from a transverse section cut half-way up the same seed. The bundles, *v. b.*, *v. b.*, are connected, as usual, by the bundle-ring (*b. r.*), from which at places an inner ring (*i. r.*) has separated; *mg*, megaspore wall. (*b. r.* + *i. r.* = *i. s.*)

U. C. L. Collection, R. 6, *b*. $\times 60$. (See p. 203.)

Fig. 32.—Papillæ from a ridge near the micropyle of a small seed.

W. 1931, A. (small seed). $\times$ about 400. (See p. 206.)

Fig. 33.—Longitudinal section through a full-sized seed showing continuity of seed-base with the pedicel and insertion of the cupule. *p*, pedicel; *v. b.*, vascular bundle of pedicel; *c*, *c*, cupule; *gl*, *gl*, glands on pedicel and cupule; *a. l.*, abscission-layer; *ch*, *c*, chalazal cushion; *pal*, palisade-layer. See also photo. 15 and 16.

S. 558. $\times$ about 15. (See p. 217.)

Fig. 34.—Median longitudinal section of a young seed or ovule, showing continuity of seed, pedicel, and cupule. The irregular black, knob-like layer is the surface of the testa, curiously preserved. The black pipes in the interior of the seed represent sections of the collapsed and folded megaspore and nucellus. *p*, pedicel; *c*, *c*, cupule; *l*, long lobe of cupule; *gl*, *gl*, glands; *h*, tuft of uniseriate hairs on long lobe of cupule; *ch*, chalaza, somewhat above position of abscission-layer; *pal*, young palisade-layer; *i. s.*, collapsed megaspore and nucellus; *p. c. w.*, pollen-chamber wall; *c*, *c*, central cone of pollen-chamber; *o*, orifice of pollen-chamber; *s*, sinus; *v. b.*, *v. b.*, vascular bundles; *a*, point of insertion of bundle to cupule.

U. C. L. Collection, R. 13. $\times$ about 33. (See p. 213.)



1



2

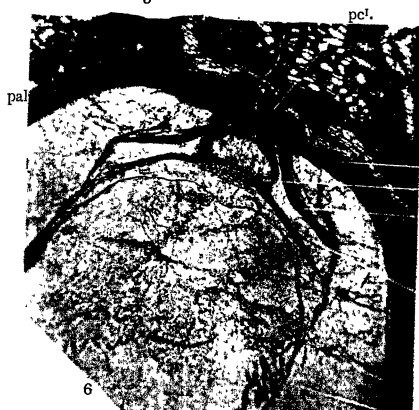


3



5 mg.

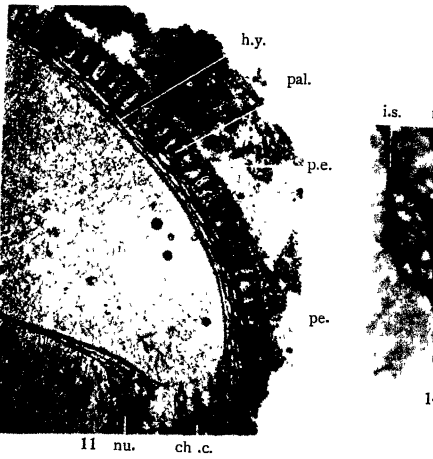
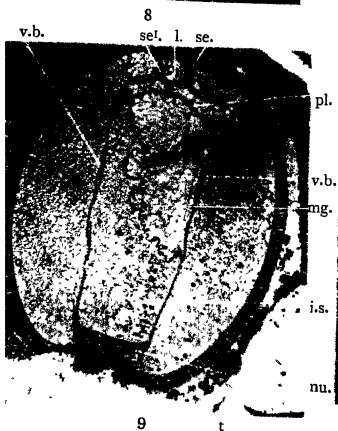
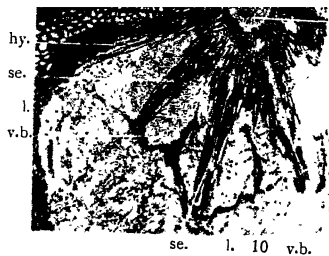
ch.c.



6



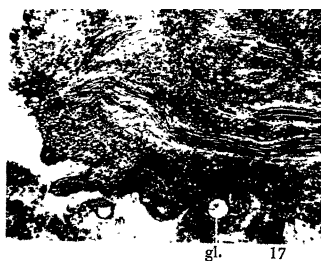
7





16

ch.c.

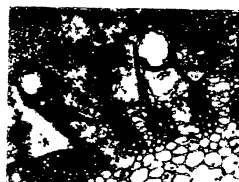


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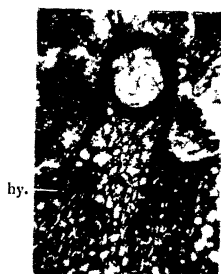
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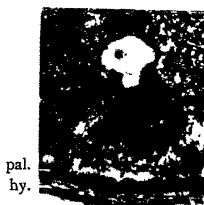
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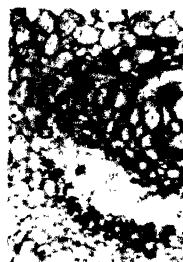
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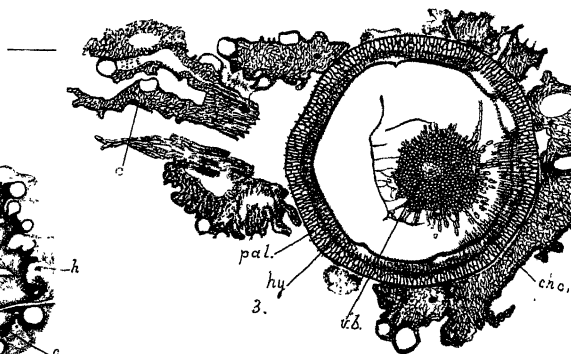
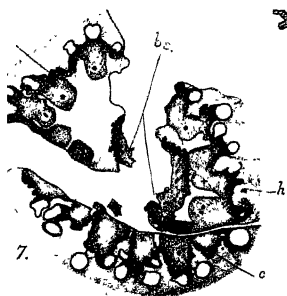
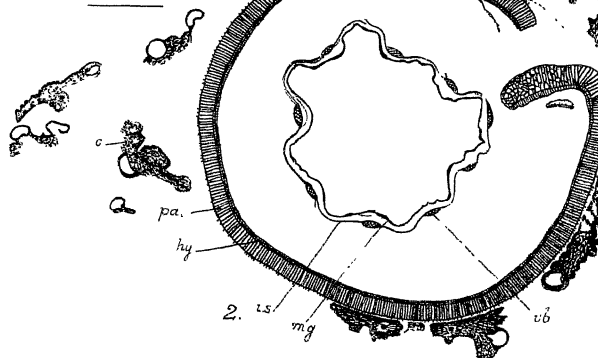
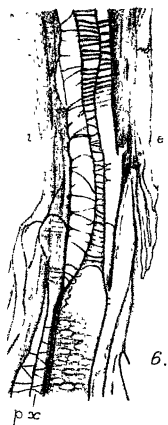
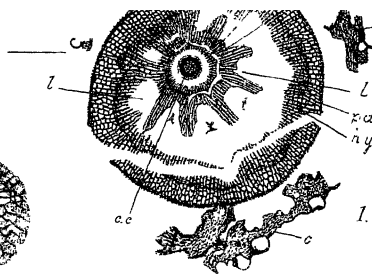
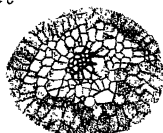
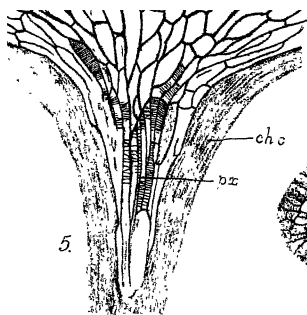


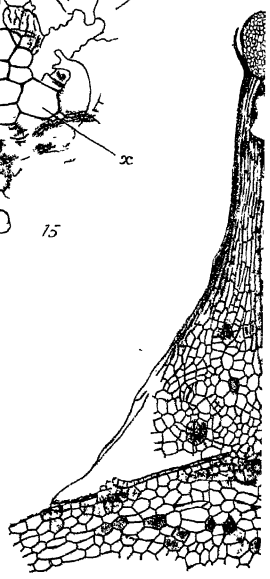
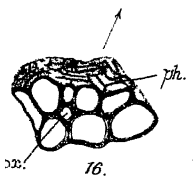
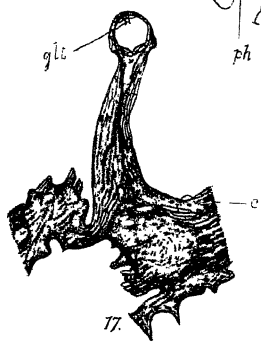
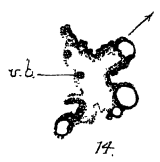
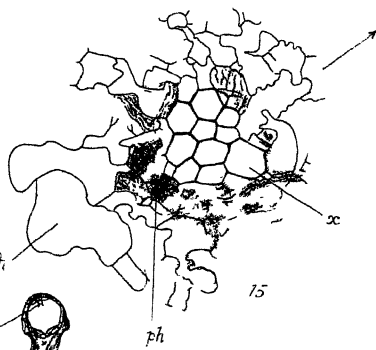
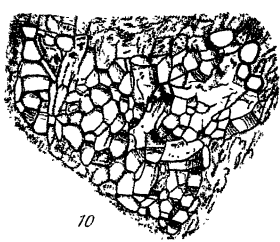
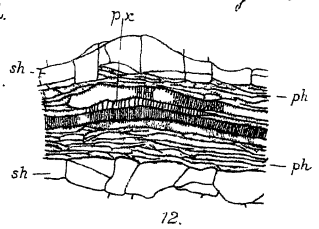
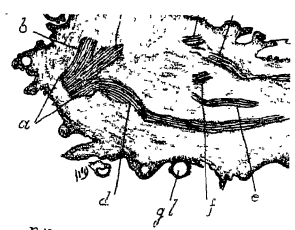
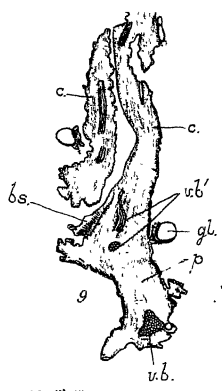
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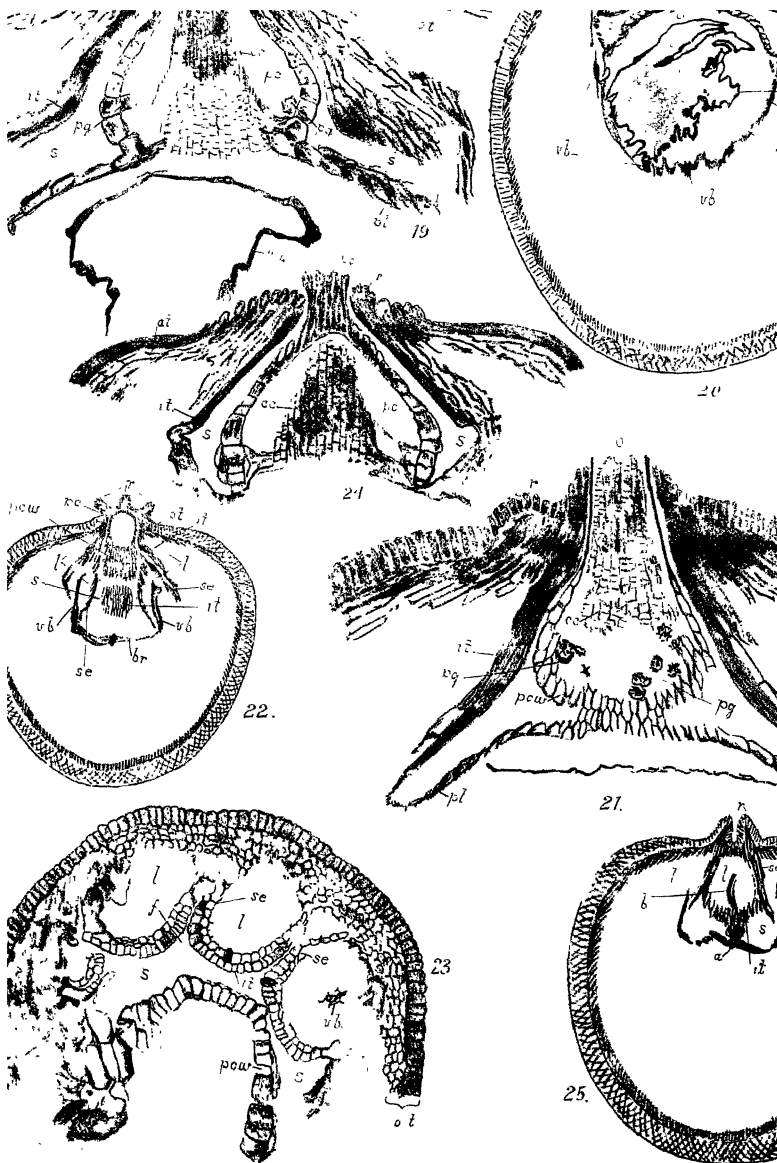
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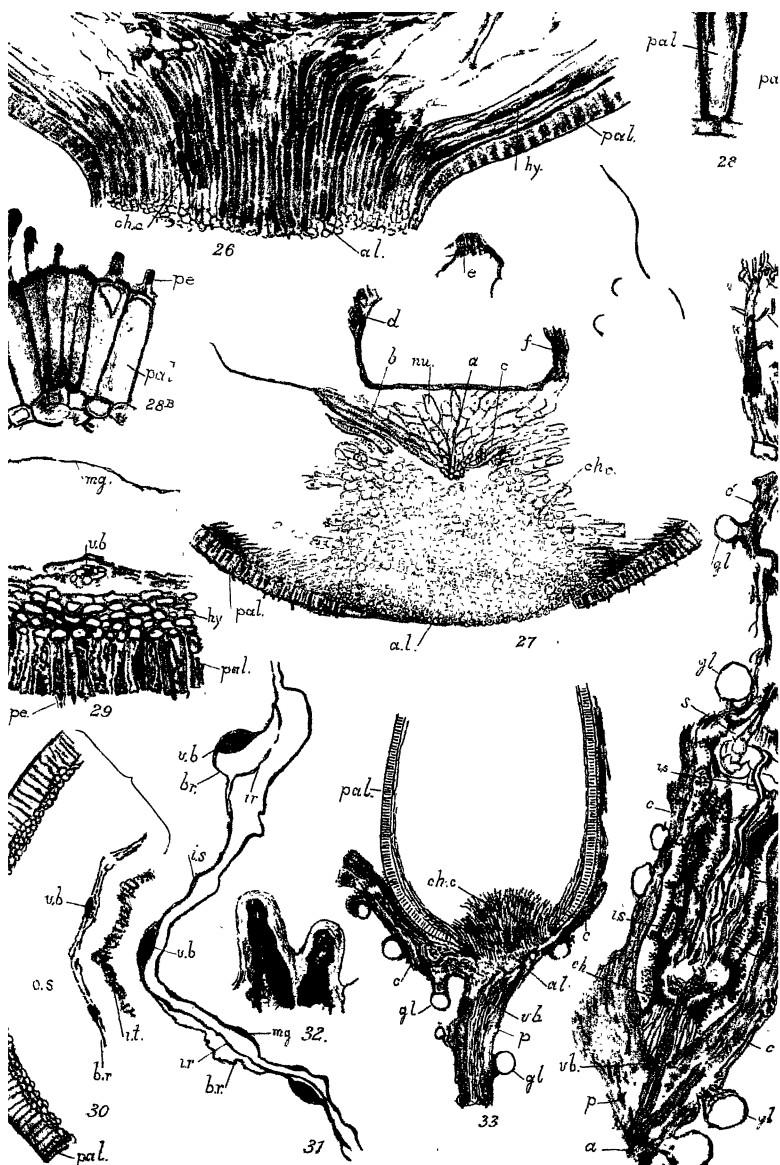
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FRASER, (Sir) T. R., and ELLIOT, (Major) R. H.
Phil. Trans., B, vol. 197, 1904, pp. 249-279.

Enhydrina Venom—its Action on the Blood, Circulation, Motor Nerves, and Respiration.
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Phil. Trans., B, vol. 197, 1904, pp. 249-279.

VIII. *Contributions to the Study of the Action of Sea-snake Venoms.*—Part I.
Venoms of Enhydrina valakadien and Enhydris curtus.

By Sir THOMAS R. FRASER, M.D., F.R.S., *Professor of Materia Medica in the University of Edinburgh, and* Major R. H. ELLIOT, I.M.S., *on Special Duty for Snake Venom Research under the orders of the Secretary of State for India.*

(From the Pharmacology Laboratory of the University of Edinburgh.)

Received May 10,—Read June 9, 1904.

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Introductory.

THE following paper is the first of a series of articles which we hope to publish on the Action of Sea-snake Venoms. The work dealt with herein was carried out mainly in the Materia Medica Laboratory of the University of Edinburgh. The kymographic work, however, was done in the Physiology Laboratory of that University, and we desire to express our sense of indebtedness to Professor E. A. SCHÄFER for permitting us to use his apparatus.

Previous Literature of the Subject.

This is very scanty. The main contributions have been the recent ones by Captain LEONARD ROGERS, I.M.S., to which we shall have frequent occasion to refer. They appear in the 'Proceedings' of the Royal Society, May 7th, 1903, and June 18th, 1903.

We may observe that the plan of the present work had been laid down by us long before we were aware that Captain ROGERS was engaged on similar lines, and that we have but very little departed from our original scheme since reading his papers. We need not say that we have read these papers with great interest.

Species of Snakes used.

The venom used in this research was obtained for us by Dr. P. P. PINTO, of Madras, who was put on special duty for this and kindred purposes by the Government of Madras, whose kind assistance we desire gratefully to acknowledge. Two species only were used, viz., *Enhydryna valakadien*, and *Enhydryis curtus*. The former is said by Dr. PINTO to be the commonest Sea-snake infesting the coast of Madras Presidency. Most of the specimens were small. The venom collected from six snakes, averaging 2 feet 6 inches in length, amounted to between 30 and 40 milligrammes, but owing to an unfortunate accident the exact amount cannot be stated. The *Enhydryis curtus* proved a comparatively rare snake. From eight specimens, Dr. PINTO obtained '022 gramme of poison or '00275 gramme per snake. The *Hydrophis gracilis* was the only other moderately large Sea-snake brought in by the fishermen, but it was too rare to enable any useful amount of venom to be collected.

We are greatly indebted to Mr. EDGAR THURSTON, Superintendent of the Madras Government Museum, and to Mr. RAMUNI MENON, of the Presidency College, Madras, for the kind assistance they gave Dr. PINTO in the work of identification.

Method of collecting the Venom.

The snakes were brought to Dr. PINTO by the fishermen, who frequently catch them in their nets along with their fish. The glands were removed with aseptic precautions, and if they were sufficiently large the venom was expressed from them into a watch glass and dried in a desiccator over sulphuric acid. The smaller sacs were dried in a similar manner and sent home in sealed bottles. They were here again dried over sulphuric acid in the exhausted receiver of an air pump. After carefully removing all tissues adhering to them, the contained venom was extracted by first opening up and minutely sub-dividing the glands, macerating them in successive small quantities of thymol water, and filtering the solutions, which were

afterwards mixed together and evaporated to dryness, over sulphuric acid, in the exhausted receiver of an air pump. There was thus obtained a yellow, brittle and scaly product, freely soluble in water and having much the same appearance and characters as the venom expressed from the fresh glands. This product, however, necessarily contained other substances than venom; but it may be noted that maceration of the glands in water produced perfectly clear solutions and very little swelling and softening of the particles of undissolved substances, unlike the hazy solutions with swollen and softened undissolved particles, which are obtained when the poison glands of the Cobra and of many other serpents are similarly treated. By this treatment, 60 dry glands of the *Enhydrina valakadien*, weighing 1·09 gramme, yielded ·28 gramme of impure venom, whose lethal power will be dealt with presently.

Appearance of the Venom.

The venom of both species, as we received it from India, consisted of thin scales of a very pale yellow colour. It readily dissolved in RINGER's fluid, which was the solvent and vehicle we employed.

The M.L.D. of Enhydrina Venom.

ROGERS (*loc. cit. supra*) estimated the potency of the mixed Sea-snake venoms employed by him "by means of numerous experiments carried out with the mixed dried venom of a number of these snakes." He found that the M.L.D. for rats was ·00007 gramme per kilo. He appears to have made only two experiments on rabbits. In one of these, ·00004 gramme per kilo. killed in about four hours, in the other, ·00001 gramme per kilo. produced no symptoms but loss of appetite, but a second dose of ·00002 gramme per kilo., five days later, killed in a few hours.

We estimated the M.L.D. of the two kinds of venom at our disposal quite separately. That of *Enhydrina valakadien*, being in the greater quantity, was tested first. The accompanying tables (I. to VI.) show the results obtained :—

TABLE II.—Estimation of the M.L.D. of the Venom of *Enhydrina valakadien* for Rabbits.

(The effect of the venom on the nerve-ends is also shown.)

Rabbit's weight in kilos.	Dose per kilo. in grammes.	Results as to life.	Effect on nerve-ends.	Remarks.
1.980	.0001	Dead in 2 hrs.	Phrenic response at 260 millims. Sciatic " 260	P.M. at once. Heart distended with blood, and still flickering.
1.990	.000075	" 2½ hrs.	Phrenic " 220 Sciatic " 190	Heart still flickering, and distended with blood.
2.115	.00007	" 4 hrs.	Phrenic " 400 Sciatic " 180	Heart in diastole; it stopped soon after the chest was opened.
2.005	.00006	" 4 hrs.	Phrenic " 400 Sciatic " 250 Vagal " 120	—
1.650	.00005	Dead in 5 hrs. 50 mins.	Phrenic " 250 Sciatic " 240 Vagal " 130	—
1.260	.00005	Ill for one day. Recovered. Killed on 6th day.	Phrenic " 450 Sciatic " 330 Vagal " 180	Lost .140 kilo. in the 6 days after injection.
2.275	.00004	Very ill for one day. Recovered.	—	—
2.345	.00003	Ill for one day. Recovered.	—	—
1.610	.000025	Recovered. Never very ill.	—	—

Note.—The nerve responses are stated in millimetres of distance of the secondary from the primary coil. The NEAVE'S hammer was used, and short shocks were given by sharply opening and closing a key in the primary circuit. The tongue could appreciate the interrupted current, when the shocks were continued for some time at 215 millims. The phrenic nerves were always tested first, then the vagi, and lastly the sciatics.

TABLE III.—Estimation of the M.L.D. of the Venom of *Euhydriua valakadun* for Cats.

(The effect of the venom on the nerve-ends is also shown.)

Cat's weight in kilos.	Dose per kilo. in grammes.	Results as to life.	Effect on nerve-ends.	Remarks.
1·980	001	Dead in 50 mins.	Phrenic response at 360 Sciatic " 340 Vagal " 110	P.M. at once. Heart beating. Blood very dark. Heart in diastole.
2·090	·0005	2 hrs.		
2·770	·00025	2½ hrs.	Phrenic 320 Sciatic 300 Vagal 60	
2·395	·00015	Recovered, but was very ill. It seemed to be moribund 6 or 7 hrs. after the injection, but had pulled round by next morning.		
2·490	·00010	Recovered.		
3·030	·00009	Recovered.		

Note.—The nerve responses are stated in millimetres of distance of the secondary from the primary coil. The NEAVE's hammer was used, and short shocks were given by sharply opening and closing a key in the primary circuit. The tongue could appreciate the interrupted current, when the shocks were continued for some time at 215 millims. The phrenic nerves were always tested first, then the vagi, and lastly the sciatics.

This series of experiments could not be completed, as the supply of venom had become exhausted. The M.L.D. for the cat is apparently ·0002 gramme per kilo.

White Rats.—It will be seen that a dose of ·00009 gramme per kilo. killed in 5 hrs. 55 mins., all larger doses causing death with proportional celerity, whilst no dose less than ·00009 gramme per kilo. proved fatal, though doses from ·00008 to ·00005 gramme per kilo. produced well-marked effects.

Rabbits.—A dose of ·00005 gramme per kilo. proved fatal on one occasion in 5 hrs. 50 mins., whilst on another occasion it merely made the animal very ill. ·00006 gramme per kilo. and all doses above this proved fatal, whilst ·00004 gramme per kilo. and all lower doses failed to kill.

Cats.—·001 gramme per kilo. killed in 50 mins.; ·0005 gramme per kilo. killed in 2 hrs.; ·00025 gramme per kilo. killed in 2½ hrs.; and ·00015 gramme per kilo. reduced the animal to such a state that for 6 or 7 hours it seemed moribund,

but by the next morning it had practically recovered. Doses of '0001 and of '00009 gramme per kilo. failed to kill. As the stock of pure venom at our disposal had now become exhausted, we were unable to continue farther, but it is evident that '0002 gramme per kilo. might with every probability of accuracy be put down as the M.L.D. for the cat.

It will be observed that our estimations of the M.L.D.'s of *Enhydrina valakadien* venom agree fairly well with those of ROGERS for rats, and with his fragmentary estimates for rabbits. In both cases his dose is lower than ours. Both he and we find the dose for the rabbit lower than that for the rat. As to the latter fact, it is partly explainable by the difficulty of getting the rabbit weighed food-free, and this, obviously, would raise its apparent weight and so lower the apparent M.L.D. This difficulty is not so great with the rat, whose real weight can be got fairly nearly after 24 hours' starvation, especially as this animal always micturates when handled.

ROGERS collected his venom by getting the snakes to bite through indiarubber sheeting; ours was obtained by expression from the dissected glands. Whilst our method facilitates the procuring of a perfectly aseptic product, it appears to yield a venom diluted with mucus, and consequently of a lower toxicity. We have observed a similar difference in specimens of Cobra venom obtained by these two methods.

It is of interest to compare the lethality of Cobra venom with that of the *Enhydrina valakadien*. The venoms were in both cases obtained for us by DR. PINTO, under the same conditions and by the same methods, and the experiments were also made under exactly parallel conditions. The results are shown in the accompanying table (IV.).

TABLE IV.

Animals used.	M.L.D. of Cobra venom.	M.L.D. of Enhydrina venom.
Rats	'0005 gramme per kilo.	'00009 gramme per kilo.
Rabbits	'0006 " "	'00006 " "
Cats	'010 " "	'0002 " "

It will be observed that the relative lethality of the two venoms for the various kinds of animal experimented with differs widely. Taking the lowest M.L.D. in each case as the unit, we obtain the following relative values for lethality :—

Cobra venom rats = 1 ; rabbits = 1·2 ; cats = 20.

Enhydrina venom (valakadien) rabbits = 1 ; rats = 1·5 ; cats = 3·3.

These figures, and especially the cat-rodent relationship, suggest most strongly that the two venoms differ materially from each other in their actions.

As our stock of *Enhydrina valakadien* venom, obtained by compression of the glands in the fresh state, had failed, we were obliged to fall back on the dried glands we had received, the venom from which was extracted in the manner already described.

The lethal power of the venom so obtained was ascertained for rats and rabbits. The following table shows the results of our experiments:—

TABLE V.

Animal used.	Weight.	Dose per kilo.	Result.
	kilos.	grammes.	
Rat	·152	·00009	Never very ill.
	·120	·0001	No effect observed.
	·145	·00015	Slight symptoms.
	·140	·0002	Found dead 19 hrs. afterwards.
	·090	·00025	Dead in 5 hrs. 40 mins.*
Rabbit	1·320	·00005	No symptoms observed.
	2·030	·000075	Decidedly ill, but recovered.
	1·217	·0001	Very ill on day of injection. In 30 hrs. the heart rate was 18, and the respiration rate 13 in 10 secs., and the breathing was laboured. The animal then slowly recovered.
	1·870	·0001	Found dead 19 hrs. afterwards.
	1·680	·00015	7 hrs. after the injection the animal was seriously ill, but not paralysed. 19 hrs. after the injection found dead and stiff.

The lethal power of this venom-extract of the dried glands is therefore a little less than half that of the purer specimen of venom expressed from fresh glands by Dr. PINTO. It may, however, be noted that a purer and much more active venom than the above could readily be obtained from dry glands, as one of us has shown, if the glands are subjected to a smaller number of successive extractions with thymol water than was adopted in the present case.

The M.L.D. of Enhydris curtus Venom.

A Belgian hare, weighing 2·34 kilos., was given ·00006 gramme per kilo. of *Enhydris curtus* venom, but only slight symptoms were produced.

A cat, weighing 2·265 kilos., received ·0002 gramme per kilo. of the venom, and it also was only slightly affected.

It was therefore obvious that the M.L.D.'s of our specimens of this venom and of that of *Enhydrina valakadien* were not the same. Our stock being limited, we selected white rats as the subjects on which to fully determine the lethality of the venom under consideration. The results were as follows:—

Rats recovered from doses of ·00007 gramme per kilo., ·00008 gramme per kilo., ·00009 gramme per kilo., ·0001 gramme per kilo., ·00015 gramme per kilo., ·00025 gramme per kilo., ·0003 gramme per kilo., ·0004 gramme per kilo., and on two occasions from a dose of ·0005 gramme per kilo.

* The left phrenic nerve responded at 420 millims., the left vagus at 40 millims., and the left sciatic at 290 millims.

A dose of .0005 gramme per kilo. proved all but fatal to a rat weighing .275 kilo. The animal lay for several hours apparently moribund. The next day it was much better, and it then steadily recovered.

Higher doses killed. The following are the details :—

TABLE VI.

No.	Weight of rat. kilo.	Dose per kilo. gramme.	Result.	
			hrs.	mins.
1	.135	.0006	Dead in	3 12
2	.125	.0007	"	1 9
3	.137	.0008	"	1 0
4	.233	.0009	"	2 18
5	.280	.001	"	1 25
6	.259	.002	"	0 58
7	.270	.003	"	0 46

The state of the nerve-ends was tested, when possible, by means of the secondary current. The following results were obtained; the distance of the secondary coil from the primary being stated in millims.

TABLE VII.

No. of experiment.	Distance of the secondary coil at which a response was obtained with the electrodes applied to		
	Phrenic nerve.	Sciatic nerve.	Vagus nerve.
	millims.	millims.	millims.
	370	280	50
	450	—	Complete inhibition not obtained with maximal stimulation, but slowing at 120.
	300	—	80
	400	—	No complete inhibition with the maximal stimulus available.

In all the above experiments, the injections were made subcutaneously at the abdomen, and absorption was hastened by rubbing the part. The venom was dissolved in RINGER'S fluid, and the bulk of each injection was about 1 cub. centim.

These results indicate that the specimen of *Enhydryis curtus* venom with which we were working was of considerably lower lethality than that possessed by our specimens of *Enhydryna valakadien* venom, a dose of .00009 gramme per kilo. of the more

powerful of the latter having proved fatal, as against one of '0006 grammes per kilo. of *Enhydryis curtus* venom.

Whilst recording this result, we do not desire to lay any great stress on it, although both venoms obtained from fresh glands were, to the best of our belief, collected under precisely similar conditions. We have found a similar discrepancy between the lethal power of different specimens of Cobra venom expressed for us from fresh glands, under conditions which, apparently, were identical with each other.

Symptoms, &c., of Sea-Snake Poisoning.

ROGERS has given an excellent description of the symptoms produced by Sea-snake venom in the lower animals. We have only two comments to make on the subject.

1. ROGERS says that it is impossible to distinguish between the symptoms in an animal poisoned with Sea-snake venom, and one poisoned with Cobra venom. We have, however, observed that in the former the urgency of the dyspnoea is much greater than in the latter. The animal (rat, rabbit, or cat) rushes from place to place as if suffocated, instead of remaining nearly motionless as a cobraised animal does. The explanation of this difference becomes a simple one, when it is realised that, as we shall afterwards show, in Sea-snake poisoning, the venom acts on the respiratory apparatus alone, and leaves the heart and blood vessels practically unaffected, in so far as any direct action on them is concerned. One of us has recently pointed out in a communication to the Royal Society that Cobra venom, on the other hand, has a powerful action on the heart: (a) directly on its muscle fibres or on the nerve-ends therein; (b) through its vago-inhibitory apparatus; and (c) through the increased pressure against which the organ has to work, in consequence of the action of the venom on the arterioles. Consequently, though death takes place in cobraism primarily through the respiratory system, there is a steadily progressing depression of the cardiac functions going on *pari passu* with the respiratory embarrassment. It naturally follows that the dyspnoea is masked thereby.

2. One of us had observed that in frogs poisoned with Cobra venom, the lower eyelid invariably rises, and that the extent of this rise is a distinct indication of the intensity of the poisoning. A similar symptom is noticed in cobraised human beings, and was recorded also by one of us as being of constant occurrence in cobraised monkeys. We refer to lid-drop of the upper eyelid. A poisoned monkey will throw back his head to obtain sight through the narrow slit left below by the increasing ptosis, and will thus assume a characteristic position. Dr. F. H. A. MARSHALL, whom we consulted on the subject, very kindly informed us that we were correct in inferring that the lower lid of the frog is depressed by muscular effort, and raised by elastic rebound on the cessation of such effort. It is of interest to note that no

such symptom is observed when an animal is dying from Daboia poisoning; indeed, it is easy to pick out frogs poisoned by Cobra and Daboia venom from each other by attention to this symptom alone. It is therefore of special interest to find that this lid-rise is of invariable occurrence in frogs poisoned by the venom of the Sea-snakes we have worked with. The point allies Cobra with Sea-snake venom in action, and distinguishes these venoms from the Daboia venom, if not also from the venom of all other Viperine snakes. We may add that the same sign has been noticed in yet another form of Colubrine snake poisoning.

It only remains to add that a frog which received '002 gramme of *Enhydris curtus* venom per kilo. died in 18 hrs.; one which received '001 gramme of the venom per kilo. died in 50 hrs.; and a third which received '0005 gramme per kilo. survived the injection 3 days, and then died. The symptoms of the poisoning were indistinguishable from those met with in cobraised frogs, failure of pulmonary respiration being an early and constant phenomenon.

The Antagonism between Calmette's Serum and Enhydris Venom.

ROGERS found CALMETTE'S serum "of no use against the poison of the Hydrophidæ.*" He tested it by adding minimal and slightly supra-minimal doses of the poison of Enhydrina to $\frac{1}{2}$ cub. centims. of fresh (CALMETTE'S) serum; white rats being used in the experiments. Whilst accepting the conclusion that the antivenene was, for all practical purposes, powerless against Enhydrina venom, we desired to satisfy ourselves as to the existence or otherwise of even a small measure of antagonism. We accordingly devised two parallel series of experiments. The first was undertaken with Cobra venom in order to standardise the sample of antivenene with which we were working; the second was carried out with the poison of *Enhydris curtus*. M. CALMETTE most kindly furnished us with a specimen of his antivenene, which was received in August, 1903, and used in February, 1904, i.e., 6 months later. The M.L.D. of the specimen of Cobra venom used had been very carefully determined for white rats and was '0005 gramme per kilo. The Sea-snake venom used was of an almost equal lethality, since a dose of '0005 gramme per kilo. reduced a rat to a moribund condition for several hours, while a dose of '001 gramme per kilo. killed in 1 hr. 25 mins.

The doses of venom and of serum were carefully calculated per kilo. weight of each animal; they were mixed in small conical glasses and allowed to stand for exactly 30 mins.; they were then injected each into the corresponding animal, and the subcutaneous tissue of the abdomen was the site selected for the injections. The tables are self-explanatory.

* 'Roy. Soc. Proc.,' vol. 71, No. 475, March, 1903.

TABLE VIII.—To test the Measure of Antagonism of Antivenene against a definite Sample of Cobra Venom of known Lethal Power.

No.	Weight of animal (white rat).	Dose per kilo. of cobra venom.	Dose per kilo. of antivenene.	Result.
1	kilo. ·183	10 × M.L.D. or ·005 gramme	cub. centims. 2	Dead in 6 hrs. 8 mins.
2	·175	Ditto	3	Found dead 18 hrs. after injection.
3	·188	Ditto	4	Ditto.
4	·166	Ditto	5	Recovered. Never really ill.
5	·087	Ditto	Nil (control)	Dead in 1½ hrs.

TABLE IX.—To test the Existence of any Measure of Antagonism of the Antivenene against a Sample of the Venom of *Enhydrys curtus*.

No.	Weight of rat.	Dose per kilo. of venom.	Dose per kilo. of antivenene.	Result.
1	kilo. ·275	gramme. ·0005	cub. centims. Nil (control)	Appeared moribund for some hours, but recovered.
2	·155	·005 = 10 × sub-M.L.D.	4	Dead in 47 mins.
3	·150	Ditto	5	Dead in 69 mins.
4	·157	Ditto	10	Dead in 91 mins.

The order in which these animals died suggests that a feeble measure of protection was afforded by the antivenene. There is, however, a great contrast between this action and that shown by the previous table, where Cobra venom was experimented with. One of us has brought forward evidence to show that the antidotal effect of antivenene is most probably due to a chemical reaction between it and the venom it is capable of antagonising.* In accordance with this explanation of serum antidotism, the above experiments indicate that there is a chemical difference between the composition of the two venoms under comparison. From this we would not unnaturally expect to find a corresponding difference between their actions. To this point we shall return later on.

* "Immunisation against Serpent's Venom, &c.," by Professor FRASER, 'Journal of the Royal Institution of Great Britain,' 1896, and 'Nature,' April 23, 1896.

Action of Enhydrina valakadien Venom on the Blood.

At our request, the following observations on this subject were made by G. SCOTT-CARMICHAEL, M.B., Ch.B., Junior Assistant in the Department of Materia Medica, who had previously made many similar observations, and in whose accuracy we have full confidence.

(a) *Action in vivo*.—1. Red corpuscles from blood, taken immediately after death from the left side of the hearts of white rats killed by Enhydrina venom, showed in films no evidence of hæmolysis having occurred. The corpuscles were of normal size and contour and of uniform intensity of colour, and rouleaux formation was present as in health.

Serum which had separated spontaneously from this blood placed in capillary tubes showed hæmoglobin coloration to the naked eye. The fluid in all cases was of a dark venous colour.

2. Red corpuscles from the blood of rats which had received less than the minimum lethal dose of this venom likewise showed no evidence of hæmolytic change, and the serum from this blood was unstained with hæmoglobin.

The blood was of a darker colour than that of health. It was examined at different intervals after the injection in each case, one examination having been made on the day of injection and another on the following day.

(b) *Action in vitro*.—These observations were made in test-tubes and on microscope slides.

1. When the hæmolytic action on the blood of rabbits and of man was tested, it was found that no effect was produced within 18 hrs. in mixtures of blood and Enhydrina venom containing 1 of venom in 500, 1 : 1000 and 1 : 2500. With 1 : 500, however, a very slight effect was produced in 21 hrs., and with 1 : 250 in 18 hrs.; and in both cases controls with mixtures of blood and saline had not yet been affected.

2. In contrast with these extremely slight effects, when similar observations were made with Daboia venom, it was found that in solutions of 1 : 500, 1 : 1000, and 1 : 2500 this venom has a relatively strong hæmolytic action, both pigment diffusion and agglutination being distinctly produced by all of the solutions within 21 hrs., and by 1 : 500 solutions in less than 18 hrs. No hæmolysis was observable in control experiments within 24 hrs.

Action of Sea-Snake Venom on the Heart.

ROGERS applied solutions of Sea-snake venom directly to the heart of pithed frogs, and obtained "no appreciable effect" thereby. He says:—"As a few drops of a 1/1000 solution of Enhydrina poison given *per venam*, and, therefore, further greatly diluted in the circulation, is very rapidly fatal, it is evident that the poison

should produce a very marked action on the heart when directly applied to it if the lethal effect is in any degree due to cardiac paralysis."

Whether these conclusions be right or wrong, we considered that they were founded on insufficient data, since FAYRER and BRUNTON* pointed out about 30 years ago that there was a great difference in the action of a presumably allied poison, viz., that of the Cobra, according as it was applied to the surface of a frog-heart or perfused through its cavity.

In a recent contribution to the pharmacology of Cobra venom,† one of us has shown that a solution so weak as 1/10,000,000 of this poison exerts an appreciable effect on the isolated frog-heart, when perfused through its cavity, and that a solution of 1/100,000 takes but a few minutes to paralyse that organ in a condition of extreme systole. When a 1:1000 solution of the same venom was dropped continuously on a suspended frog-heart, only a very slight quickening of the beat and a slight tendency of the organ to pass towards systole was observed. The contrast between these two sets of results is very marked, and confirms FAYRER and BRUNTON's work. We were, therefore, of opinion that it was not possible to definitely decide that Sea-snake venom had no cardiac action till experiments had been made both with the isolated heart and with the heart exposed *in situ*. It seemed important to make both sets of experiments, since previous work with Cobra venom had shown that the heart is acted on by that poison in two manners; on the one hand, by the direct systole-increasing action of the venom on the muscle tissue or on its nerve-ends, and, on the other, by the tendency to inhibition caused by the direct action of the venom on the vago-inhibitory centre as well as by its indirect action on the same centre through the medium of failing respiration. Our first object was to ascertain if either or both of these actions was likewise exerted by Sea-snake venom.

Perfusion of the Frog-Heart with Solutions of Enhydrina Venom.

A modification of SCHÄFER's plethysmograph was employed for this purpose. The details of the experimental method were in all respects the same as those described by one of us† in a similar series, in which Cobra venom was under investigation. The fluid perfused consisted of one part of ox-blood and two parts of RINGER's fluid.

Experiment 1.—An isolated frog-ventricle was perfused with a 1:20,000 solution of Enhydrina venom, with negative results.

Experiment 2.—An isolated frog-ventricle was perfused with a 1:5000 solution of Enhydrina venom. The beat quickened slightly, and the heart passed into fatigue in the ordinary course.

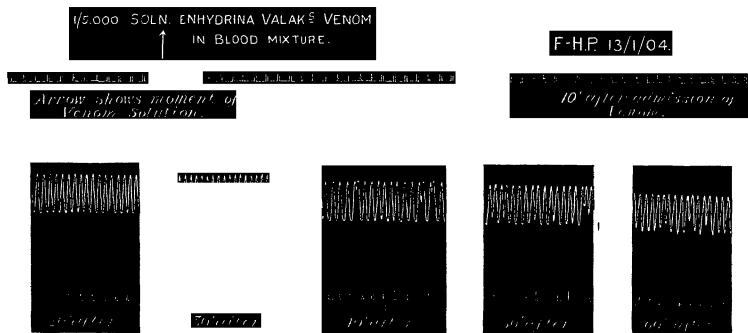
* 'Roy. Soc. Proc.,' vols. 21 and 22.

† 'A Contribution to the Study of the Action of Indian Cobra Poison,' by Captain R. H. ELLIOT, communicated to the Royal Society, January 18, 1904.

Experiment 3.—Was undertaken to make sure that the quickening observed was actually due to the venom. A heart was perfused for 2 hrs., and whilst its beat was very steady a 1 : 5000 solution of Enhydrina was admitted. The following were the changes observed in the heart's rate :-

4 mins. before venom was admitted there were	21.5 beats per min.
3 " "	21
2 " "	21
1 min. "	21.5
1 min. after	21.5
2 mins. "	22.5
3 " "	22.5
4 " "	22.5
5 " "	23
6 " "	24
7 " "	24.5
8 " "	25
9 " "	25.5
10 " "	25
20 " "	23

Parts of the tracing are also shown.



ROGERS* found that the average quantity of dried venom obtained from single bites of thirteen different living specimens of the Enhydrina was just under 1 centigramme. It will be remembered that our own estimates of the available

* 'Roy. Soc. Proc.,' vol. 71.

amounts of venom, in the glands of the species we have had to do with, were much lower than this.

Accepting ROGERS' figure of about a centigramme of venom per bite, the maximum possible concentration in the blood of an average man would be about 1 : 500,000. This figure is arrived at by taking the weight of an average man at 70 kilos, and his blood at about one-fourteenth of this figure. The calculation stands thus : 70,000 grammes $\times \frac{1}{14} \times \frac{1}{100} = \frac{1}{500,000}$.

Since a solution 100 times as strong as this has but very feeble action on the isolated ventricle, whilst the results of perfusion with a solution twenty-five times as strong as that which could be in operation after a bite are negative, we may safely conclude that *Enhydrina* venom differs markedly from Cobra venom, in that it has no direct action on the heart in any strength of solution ever likely to be present in the bodies of human victims of this species of Sea-snake.

Perfusion of the Vessels of the Frog with Enhydrina Venom.

Two experiments were made with a solution of *Enhydrina valakadien* venom of a strength of 1 : 50,000. The results were entirely negative.

It is of interest to recall, as one of us has shown,* that Cobra venom, even in a solution so dilute as 1 : 10,000,000, produces decided constriction of the vessel walls. The contrast between this powerful action and the absence of direct constriction by *Enhydrina* venom has a distinct bearing, as might have been expected, on the level of blood-pressure in these two different kinds of snake poisoning. This point will afterwards be returned to.

We are indebted to Mr. JOLLY, who carried out these experiments for us under our supervision.

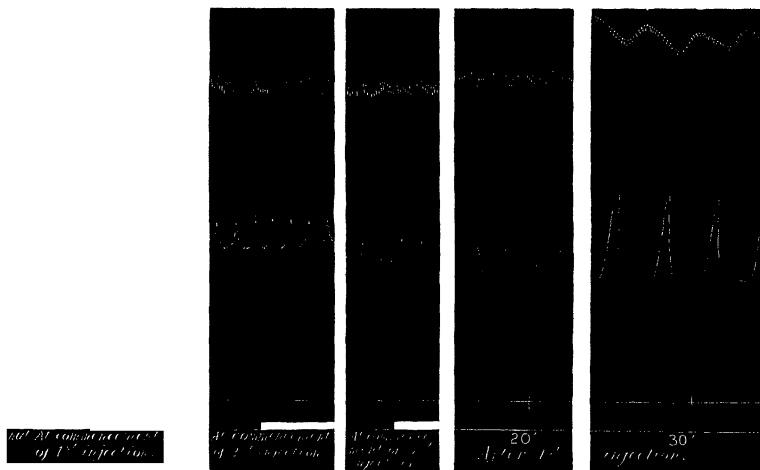
Kymographic Experiments with the Venom of Enhydrina valakadien.

Experiment 1.—A cat, weighing 2·5 kilos., was etherised and a tracheal cannula inserted, through which ether was then given; respirations were recorded by means of a double stethograph; the blood pressure was taken in the carotid; a cannula was tied into the external jugular; and the venom solution was placed in a graduated cylinder connected with the cannula by a short piece of rubber tubing. On this tubing a clip was placed, by the removal of which the solution could be admitted, and the fluid thus found its way slowly into the heart by its own weight, or, if required, it could be introduced faster, by means of a rubber ball arrangement which screwed on to the top of the cylinder.

After a normal had been obtained, one-fifth of the S.M.L.D. (subcutaneous minimum

* Captain R. H. ELLIOT, I.M.S., 'A Contribution to the Study of the Action of Indian Cobra Poison,' communicated to the Royal Society, January 18, 1904.

lethal dose) was introduced into the circulation, dissolved in 10 cub. centims. of RINGER's fluid. The injection lasted $2\frac{1}{2}$ mins. A distinct rise of blood pressure took place, and continued after the injection had been finished; but the rate and force of respiration did not much alter. 6 mins. after the commencement of the first injection, a second injection was made, which occupied 3 mins. Very little effect was produced, and 12 mins. after the commencement of the first injection a third was given, equivalent to 4 M.L.D.'s, in 20 cub. centims. of RINGER's fluid, and this took 6 mins. to pass in, and was accompanied first by a slight fall and then by a very large rise of blood pressure. The rise continued after the injection had been completed, and was synchronous with the increasing failure of respiration. A fall occurred only when the heart stopped beating, or at least recording. The heart-rate remained very constant throughout the experiment (Table X.).



Experiment 1.—Extracts from Blood-pressure Tracing. Time marked = 10 secs.

Experiment 2.—A rabbit, weighing 2·17 kilos. All details of preparation were as in the previous experiment; 2 S.M.L.D.'s were given intravenously in 20 cub. centims. of RINGER's solution; the injection, which was much faster than had been intended, took 55 secs. The blood pressure rose rapidly, then fell back to its original level, but it rose again as respiration failed, and then fell sharply to death. The first rise was probably mainly due to the large bulk of the injection, and the second was obviously asphyxial. The slowing of the heart, which accompanied the respiratory failure, was also probably asphyxial, and due to an action on the vago-inhibitory centre. Respiration was rapidly paralysed by the venom (Table XI.).

TABLE X.—Experiment No. 1.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
	millims.	per min.	per min.	millims.
1 min. before injection	115	30	3	6 to 9
One-fifth subcutaneous M.L.D. of <i>Enhy. val.</i> venom in 10 cub. centims. of RINGER's fluid intravenously in $2\frac{1}{2}$ mins.				
1 min. after commencement of injection	118	27	2.5	6.5
2 mins. " "	119	27	2.25	3.5
3 " " "	118	26	3	7.5
4 " " "	118	26	3	5 to 8
5 " " "	120	26	3.25	8 to 10
6 " " "	119	27	3	7
One-fifth subcutaneous M.L.D. in 10 cub. centims. of RINGER, given in 3 mins.				
7 mins. after commencement of injection	122	26	3.25	6.5
8 " " "	119	27	3	6.5
9 " " "	118	27	3.25	6 to 8
10 " " "	117	27	3.5	6.5
11 " " "	118	27	3.5	9
12 " " "	118	27	3	5
Four subcutaneous M.L.D.'s in 20 cub. centims. of RINGER, given in 6 mins.				
13 mins. after commencement of injection	122	29	3.75	7.5 (struggle)
14 " " "	122	29	4.5	7 to 10
15 " " "	115	27.5	3.5	7.5
16 " " "	112	27	3.25	6
17 " " "	108	26	3.25	5
18 " " "	110	27	2.75	7
19 " " "	115	28	2.75	12
20 " " "	122	27	2.5	5 to 8
21 " " "	122	26	2.2	9
22 " " "	124	26	2.2	9
23 " " "	127	26	2	12
24 " " "	135	25	1.75	15
27 " " "	137	26	1.5	16.5
28 " " "	154	24	1	21
29 " " "	144	25	1.5	22
30 " " "	140	27	1.5	21
31 " " "	140	27	1.25	19.5
32 " " "	146	27	1.25	17.25
33 " " "	148	29.5 (flickering)	1	12
34 " " "	124	0	0	0



Experiment 2.—Extracts from Blood-pressure Tracing.

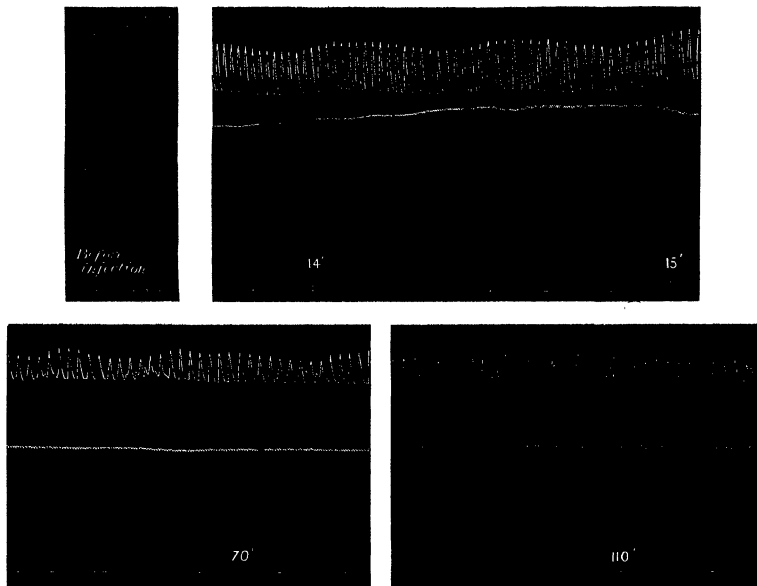
TABLE XI.—Experiment No. 2.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
$\frac{1}{2}$ min. before injection . .	millims. 105	per min. 36	per min. 23	per min. 4·5
One subcutaneous M.L.D. of <i>Endy. val.</i> venom given by external jugular in 20 cub. centims. of RINGER's fluid.				
1 min. after commencement of injection	105	36·5	12	3·25
2 mins. " "	115	37·5	10·5	3
3 " " "	122	37	10	2
4 " " "	124	36	9·5	2
5 " " "	113	35	9	2·25
6 " " "	107	32	7·75	2·5
7 " " "	104	31	6·5	2·5
8 " " "	111	30	4·75	2
8 " 40 secs. "	128	25	0	0
9 " 0 " "	108	17	0	0
10 " " "	73	10·5	0	0
11 " " "	65	11·5	0	0
12 " " "	45	12	0	0
13 " " "	45	0	0	0

Experiment 3.—A rabbit, weighing 2·18 kilos., was etherised, and a tracheal cannula inserted, through which the anæsthetic was continued to be given. Blood pressure was taken in a carotid, and respiratory movement was recorded by means of a double stethograph. Injections were made through an external jugular vein.

After taking a normal, one-fifth of the subcutaneous M.L.D. was injected intravenously, dissolved in 4 cub. centims. of RINGER's fluid. There was a sharp rise of blood pressure, followed by an equally sharp fall. At the time of the second injection the pressure had fallen till it stood at the same point as that which it had occupied at the beginning of the experiment. At the 85th minute, a second injection, the same in amount, &c., as the last, was given; and at the 105th minute, a third injection, double the dose of either of the two preceding, was given. The rises which followed each of these injections were probably largely due to the bulk of fluid injected. The heart rate remained very nearly constant for a long time and then fell slowly. Respiratory rhythm was early affected, as is shown by the accompanying extracts from the tracings. ROGERS recorded his observation "that Cheyne-Stokes breathing is sometimes simulated" in Enhydrina poisoning, experimentally produced. Our extracts from this tracing (p. 269) are an interesting confirmation of his statement. As death approached the blood pressure tended to fall. This observation is not really in opposition to ROGERS' experience of a marked rise in blood pressure ushering in death, for in all his recorded experiments death occurred much more rapidly than in the

case we are now considering. Indeed, it was our conviction that ROGERS had confined himself too exclusively to large doses, and this led us to administer much smaller doses in addition to large ones. We need hardly insist on the advisability of approximating the doses (so far as experimental requirements admit) as closely as possible to those likely to be met with in actual experience. Details of the tracing will be found in the accompanying table (Table XII.). The main inference would appear to be that the venom has no cardio-inhibitory action such as one meets with in cobraism,



Experiment 3.—Extracts from Blood-pressure Tracing, showing marked changes of respiratory rhythm.

but that the increasing venosity of the blood, which is induced by slow failure of respiration, exerts an indirect inhibitory influence on the heart through the vagal centre in the medulla oblongata. When rapid failure of respiration occurs, with a heart still undamaged, abrupt rises of blood pressure accompany asphyxiation. This is not the case in the slower cases whose course we have just sketched out above. It may be urged that in cobraism, abrupt asphyxial rises of the blood-pressure curve immediately precede death, even though Cobra venom has a marked inhibitory action on the heart, both direct and indirect. There is, however, an element in cobraism, which we have shown to be lacking in Sea-snake poisoning, viz., the action

TABLE XII.—Experiment No. 3.

Time.	Blood pressure.	Heart beats.	Respirations.	Resp. excursions.
$\frac{1}{2}$ min. before injection . .	millims. 100	per min. 30	per min. 7.5	per min. 7.5
One-fifth subcutaneous M.L.D. intravenously in 4 cub. centims. of RINGER.				
1 min. after commencement of injection	116	28	6.5	9
2 " " "	116	28	5.75	9.5
3 " " "	119	27	5.5	10
4 " " "	123	27	5.5	11
5 " " "	124	27.5	5.75	11
6 " " "	124	26	5.75	11
7 " " "	126	27.5	5.75	12
8 " " "	128	27.5	6	12.25
9 " " "	130	28	6	11.5
10 " " "	129	27.5	6	12
15 " " "	130	29	6.75	12
20 " " "	121	30	9	8
25 " " "	104	29	6.25	4.25
30 " " "	102	28	6.75	7.5
35 " " "	110	29	7	7 to 14
40 " " "	110	28	7	6 to 12
50 " " "	108	28	7	6 to 8.5
55 " " "	107	28	7	6 to 10
60 " " "	105	28	7	7 to 9.5
70 " " "	102	27	7	4.5 to 7.5
80 " " "	101	26	7	6.5
85 " " "	100	26	6.75	4 to 7.5
One-fifth subcutaneous M.L.D. intravenously in 4 cub. centims. of RINGER given in 1 min.				
90 mins. after commencement of first injection	100	26	6.75	4.5 to 6.5
95 " " "	102	25	7	4 to 6.5
105 " " "	104	25	6.25	3 to 5.5
Two-fifths of subcutaneous M.L.D. intravenously in 8 cub. centims. of RINGER given in 3 min.				
110 mins. after commencement of first injection	103	24	6	4 to 5.5
120 " " "	110	23	5	1.5
125 " " "	80	21	—	—

After this the heart slowed steadily, respiration rapidly failed and the animal was dead 23 mins. later. Blood pressure fell steadily to the end. Respiratory failure was presumably the cause of the cardiac slowing.

of the venom on the blood-vessels, which leads to powerful constriction of their walls and so maintains a high pressure, despite other opposing influences. As we have

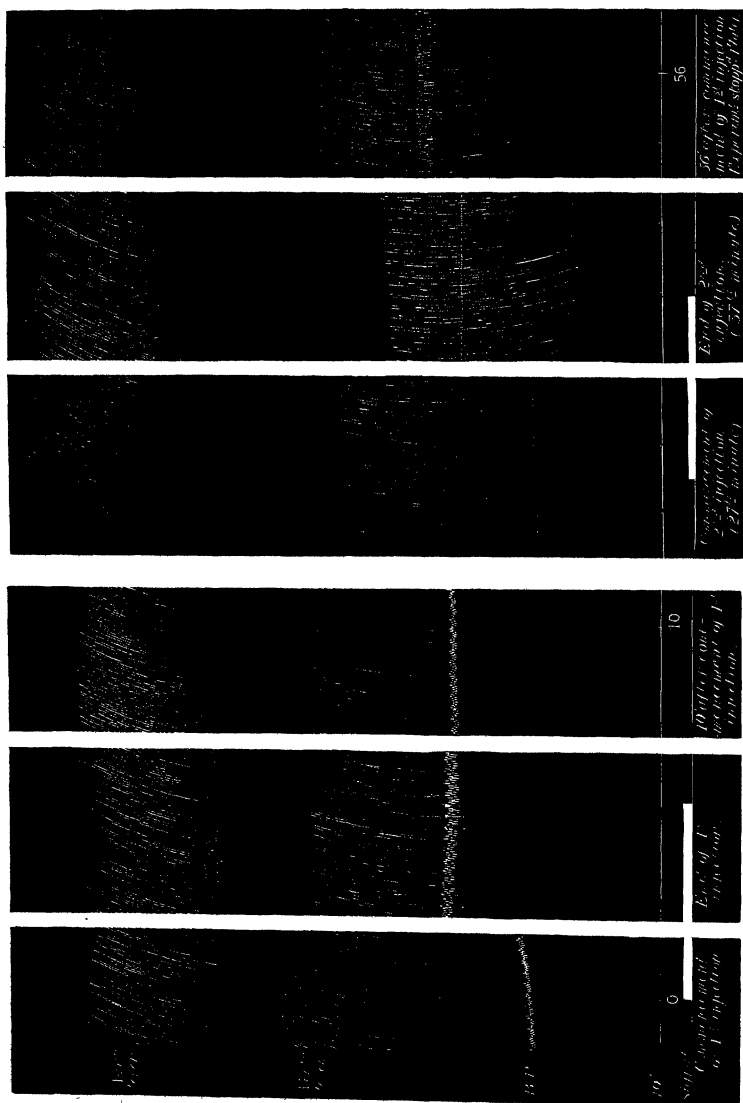
already shown, the venom of *Enhydrina* has no direct constrictive action on the arterial walls.

Experiment 4.—A cat, weighing 2 kilos., was etherised, and etherisation was maintained through a tracheal cannula. The blood pressure was taken in a carotid artery, and the auricular and ventricular movements were recorded by means of levers attached to the heart by hooks and silk threads, the chest wall being partly removed for the purpose. Two injections were made through an external jugular vein of five and ten times the subcutaneous minimum lethal dose, respectively, of *Enhydrina valakadien* venom.

The accompanying table (Table XIII.) shows the changes observed. The high rises of blood pressure after each injection are, doubtless, due to the volume of fluid injected. It will be observed that at the beginning of the second phase of the experiment, the pressure had fallen to its original level. The force of beat was lessened, but that is to be expected in operations so severe as this, especially in winter, even although, as in this experiment, all ordinary precautions to avoid shock are taken. The striking feature of the experiment is well shown on the accompanying extracts from the tracings (p. 272), and is the absence of all signs of inhibition, even though the doses of venom given were relatively very high, viz., five times the subcutaneous M.L.D. on the first administration, and ten times the subcutaneous M.L.D. on the second administration. Both the rate of beat and the amplitude of the excursions of the auricular and ventricular levers show by their constancy that this venom has no direct action on the heart, even when given intravenously in doses far above those which would be met with in snake-bite in man.

Experiment 5.—A cat, weighing 2·5 kilos., was etherised, and a cannula was placed in the trachea for the farther administration of the anæsthetic. Blood pressure was taken in the carotid, and respirations were recorded by means of a double stethograph (the movements of the lever are expressed in millimetres as some indication of the respiratory excursions). Injections were made by the external jugular vein with a fine hypodermic needle, and the venom solution consisted of ·001 gramme of *Enhydrina valakadien* venom, extracted from glands, dissolved in 1 cub. centim. of RINGER's fluid.

The subjoined table (Table XIV.) shows the results as recorded. In this experiment we were careful to reduce the bulk of our injections, and it is here clearly demonstrated that there is no rise of blood pressure apart from that attendant on progressive asphyxiation. Even then we do not notice any large rise, but, on the contrary, the pressure gradually falls towards death, the heart at the same time failing. A point of interest is the appearance, in this tracing also, of curves indicating a type of breathing like the Cheyne-Stokes type. These curves make their appearance directly after the second injection. The type of respiration is altered in another respect, as may be seen in the extracts from the tracing (p. 274). It will be observed that expiration immediately follows inspiration in the upper respiratory

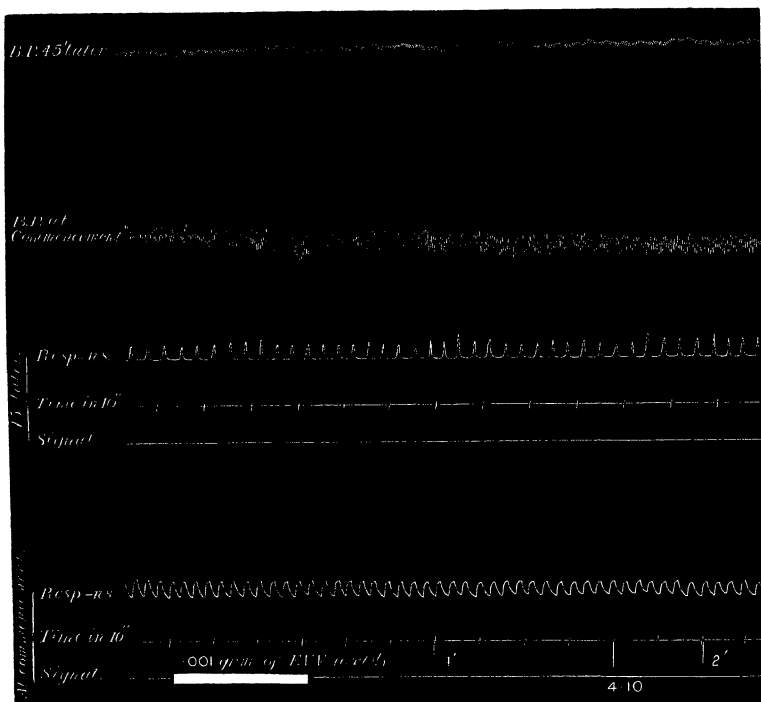


Experiment 4.—Extracts from Blood-pressure Tracing with heart exposed. Auricular and ventricular movements recorded by suspension method.

TABLE XIII.—Experiment No. 4.

Time.	Blood pressure.	Heart beats.	Auricular beat.	Ventricular beat.
	millims.	per min.	per min.	per min.
1 min. before injection . .	84	30·5	28	14·5
Five subcutaneous M.L.D.'s injected intravenously in 5½ mins. (solution '0001 gramme = 1 cub. centim.)				
min. after commencement of injection	110	30·5	29	14·5
2	117	29	29	14·5
3	122	29	28·	14·5
4	122	29	28·	14·5
5	124	29	31	14·5
6	125	29	29	14·5
7	120	29	31	14·5
8	122	29	31	14
9	122	29	31	15
10	124	29	32	15
11	127	29	29	14
12	134	29	28·	16
Manipulation to remove clot (cannula opened).				
13	126	31	33	15
14	123	30	33·5	18
15	125	31	33·5	16·5
An interval of 12 mins., during which clot had to be twice run out, and auricular and ventricular levers were readjusted.				
Second injection of ten subcutaneous M.L.D.'s was made between 27th and 37th mins.				
27 mins. after commencement of injection	86	22	22	19
28 " " "	86	22	23	19
29 " " "	93	21	26	19
30 " " "	97	22	26	19
31 " " "	102	21	23·5	19
32 " " "	104	21	27	21
33 " " "	107	21·5	27	21
34 " " "	108	21·5	27	23
35 " " "	112	21·5	28·5	24
36 " " "	116	22	27·5	23
37 " " "	119	22·5	27·5	22·5
38 " " "	120	22	25	22
39 " " "	122	24	26	22
Manipulation to remove clot, and levers readjusted.				
45 " " "	119	24	23·5	22·5
46 " " "	—	—	—	—
50 " " "	122	24	22	22
55 " " "	136	23	22·5	26
57 " " "	136	23	20	25

trace, and that the pause between successive respiratory movements is greatly prolonged at the expense of the movements themselves.



Portions of two Tracings from Experiment 5 (*Enhy. val. venom*) are here partly superimposed. The lower one is at the time of first injection, the upper one is 45 mins. later.

Action on the Motor Nerve-ends.

If a very large dose of the Sea-snake venoms dealt with in this paper is injected intravenously into an animal, the animal dies in a few minutes, and asphyxial convulsions are absent or ill-marked. ROGERS noticed this, and attributed it to the respiratory centre being completely paralysed "before the failure of the breathing had had time to render the blood sufficiently venous to produce respiratory convulsions." He adds, "Another possible explanation must, however, be considered a paralysis of the end-plates of the motor nerves might cut off the peripheral

TABLE XIV.—Experiment No. 5.

Time.	Blood pressure.	Heart beats.	Respiration.	Resp. excursions.
	millims.	per min.	per min.	millims.
Before injection	151	25	3	5
·001 gramme (2½ subcutaneous M.L.D.'s) of extracted <i>Enhy. val.</i> venom dissolved in 1 cub. centim. of RINGER'S fluid injected intravenously in 30 secs.				
1 min. after commencement of injection	149	25	3·5	4
2 mins. " "	145	25	3·5	3
3 " " "	145	26	3·5	3
4 " " "	145	27	3·5	3
5 " " "	145	27	3·5	3
10 " " "	151	28	3·5	1·5 to 7
12 " " "	151	29	4·5	1·5 to 8
Artery cannula opened to detach clot twice over, thus causing fall in blood pressure.				
20 mins. after commencement of injection	122	26	2·5	1·5
25 mins. " "	122	26	2·5	1·25
30 " " "	126	25	2·25	1·5
35 " " "	129	25	2·75	2
37 mins. after first injection, a second injection the same as the first was given.				
37 mins. after commencement of first injection	121	25	2·5	2·5
38 mins. " "	122	25	2·5	1·5 to 2·5
39 " " "	122	26	2·5	1·5 to 2·5
40 " " "	125	26	3	2·5 to 3
41 " " "	125	26	2·75	2 to 3
42 " " "	125	26	2·75	1·5 to 4
After the second injection, Cheyne-Stokes breathing became well marked.				
45 mins. after commencement of first injection	127	26	3	2·5 to 6
50 mins. " "	127	26	2·5	4 to 6
52 " " "	119	24	2	5 to 6
54 " " "	105	23	2	4·5 to 7
55 " " "	94	22	2	2·5 to 8·5
56 " " "	54	16	2	1·5 to 6·5
57 " " "	14	10	0	0
60 " " "	Dead	—	—	—

muscles from the action of the respiratory centre, in spite of its over-stimulation by venous blood." He then proceeded to test the state of the motor nerve-ends in two legs, one of which was ligatured and one free, and he showed that those of the protected leg remained active, whilst those of the other became paralysed. He concludes that "it is therefore impossible to say how far the absence of convulsions

is due to this cause (paralysis of the phrenic nerve-ends), and how far to failure of the respiratory centre. To clear up this point he made two experiments, one on a cat and the other on a rabbit. The phrenic (left) was exposed in the neck, and stimulated at intervals, the results being graphically recorded. He was led to conclude that "the first and most important action of the poison appears to be its effect on the respiratory centre, although the paralysis of the phrenics speedily ensues, and is a very important feature of the action of the venom."

ROGERS used a current with the secondary coil at 25 millims. RAGOTZI* has stated that in order to obtain evidence of peripheral nerve-end paralysis in cobraism, one must avoid the administration both of large rapidly fatal doses and of too small doses. In fact, there must be a moderately large dose in circulation, and it must be allowed a sufficient time to act. One of us has recently shown that the phrenic nerve-ends remain fully sensitive (*a*) in animals which have been poisoned by large intravenous doses of Cobra venom, and (*b*) in those which have been killed by subcutaneous doses not greatly exceeding the minimum lethal. It was at the same time shown that the phrenic nerve-ends can be paralysed by giving lethal doses and then maintaining life by means of artificial respiration. The obvious inference was that in cobraism the respiratory centre is first attacked; and that this was not improbable was demonstrated by exposing the medulla oblongata and applying Cobra venom directly to its surface, when the centre speedily became paralysed, in one case death occurring in 12 mins.

In order to investigate more fully the action of Enhydrina poison on the various nerve-ends, and especially on the phrenics, three series of experiments were undertaken.

Series 1.—Experiment 1.—A rat weighing .195 kilo. was etherised, and received an intravenous injection of *Enhydris curtus* venom. The dose was equivalent to 14 M.L.D.'s if given subcutaneously, and was dissolved in 1 cub. centim. of RINGER's fluid. Death took place in 4 mins. The chest was at once opened and the exposed phrenics were tested with the secondary current (NEAVE's hammer in circuit). No diaphragmatic contraction was observed even with a maximal stimulus. The vagi were next exposed and stimulated; again, even a maximal stimulus failed to produce a decided response, the heart being slowed but not stopped. A sciatic nerve was then tried and gave a response at 120 millims.

Experiment 2.—A rat weighing .132 kilo. received intravenously seventeen times the subcutaneous M.L.D. of *Enhydris curtus* venom, and was dead in $2\frac{1}{2}$ mins. The left phrenic nerve again gave no response to maximal stimuli; the right responded by a feeble diaphragmatic twitch with the secondary coil at 70 millims. Even with a maximal stimulation the diaphragmatic twitch was very feeble. Stimulation of the vagi, with the secondary coil home, again failed to inhibit the heart

* VIRCHOW's 'Archiv für Pathol. Anat. und Physiol.,' vol. 122, 1890, p. 201.

completely though it slowed it. The left sciatic responded at 180 millims. by producing a feeble muscular twitch.

Experiment 3.—A precisely similar experiment was made with a rabbit which received ten times the subcutaneous M.L.D. intravenously. A maximal stimulation of the phrenics produced no diaphragmatic response.

Experiment 4.—A rat was etherised and its trachea was ligatured. As soon as death had occurred, the nerve-ends were tested as before, with the following results—Phrenic response at 450 millims.; vagal response at 90 millims. Clearly, therefore, the nerve-end paresis we have noted was not accounted for by suffocation. Experiments on cats, rats and rabbits killed by a blow on the head have shown that stimulation of a normal phrenic nerve or of a sciatic with the secondary coil at 450 millims. produces marked muscular contraction. The exact strength of current to produce a complete temporary inhibition of the heart varied. In rabbits and cats such inhibition was usually got with the secondary coil at 100–140 millims., but in rats one had generally to bring the coil nearer, 80–90 millims. being a common but not always sufficiently approximated distance to cause definite inhibition.

It should be mentioned that even when the nerves of the poisoned animals above dealt with failed to give any response to a maximal stimulus, the muscles responded readily to a comparatively weak shock.

Series 2.—Whenever it was possible to do so, we tested the condition of the phrenic, sciatic and vagal nerve-ends immediately on death in the animals used for ascertaining the minimum lethal doses. In order to keep the conditions uniform, we watched each animal, and opened the thorax as soon as the corneal reflex had disappeared and respiration had ceased. We are unable to lay down any rule establishing a relationship between the amount of nerve-end paresis noted and the doses of venom used, but we found a distinct diminution of nerve-end activity so constantly present as to leave no doubt in our minds that we were dealing with cause and effect.

The direct Action of Enhydryna Venom on the Respiratory Centre.

Series 3.—We exposed the medulla oblongata of rabbits, by dividing the soft structures at the back of the neck, and cutting away the posterior occipito-atlantoid ligament. A large quantity (2–5 milligrammes) of *Enhydryna* venom was then dropped on to the medulla over the seat of the respiratory centre. The type of respiration was in each case quickly and profoundly affected, and the animals died with every evidence of respiratory failure, and the excitability of the phrenic and other nerve-ends was found at death to be distinctly impaired. We could not succeed in killing an animal with its nerve-ends still undamaged, as can be done when Cobra venom is applied in a similar way.

It would accordingly appear to be impossible with Enhydrina venom to kill a rabbit directly through its respiratory centre, before the poison has had time to enter the general circulation and thus affect the nerve-ends. On the other hand, the rapidity with which the type of respiration was altered, both in the above experiments and in those in which the venom was intravenously injected, appears to us to leave no room for doubt that the respiratory centre is directly affected by this venom. The conclusion would appear to be that both the centre and the nerve-ends are affected, and that this double action is responsible for the remarkable embarrassment of respiration which is characteristic of the action of Sea-snake venoms.

Summary of Conclusions.

(1) Enhydrina venom has no direct action on the walls of the arterioles, or at least has no such action in any strength of solution which could be met with in the blood of a human victim of Sea-snake bite.

(2) Enhydrina venom acts directly on the isolated frog-ventricle, producing a tonic and stimulating effect, but this action is produced only by very strong solutions (1:5000). The heart beat is quickened, and the result is, therefore, similar to that produced by very weak solutions of Cobra venom (1:1,000,000 or weaker).

(3) By experimenting with the mammalian heart exposed *in situ*, we have shown that Enhydrina venom has no direct action on the vagal cardio-inhibitory centre. This affords a striking contrast to the condition observed in Cobra poisoning. In the latter case, the powerful tonic and stimulant action of the venom on the heart muscle (or probably on its nerve-ends) is masked by equally powerful and direct stimulation of the cardio-inhibitory centre. In Enhydrina poisoning, on the other hand, the complete absence of cardio-inhibition leaves the feeble tonic action on the heart free to manifest itself, as appears to be displayed in several of our tracings. We cannot otherwise explain the increase in rate of the heart beats which we have not infrequently observed.

(4) Enhydrina venom has apparently no direct action on the vaso-motor centre. ROGERS found that on the injection of a Viperine venom, or of the venom of the Colubrine Banded Krait (*Bungarus fasciatus*) into an animal, a marked fall of blood pressure occurred, which was of central origin. In collaboration with Drs. SILLAR and CARMICHAEL (of the Materia Medica Laboratory, Edinburgh), one of us has found that a similar and powerful action is exerted by the venom of the common Krait (*B. caruleus*).^{*} The absence of any such action in the Sea-snake venoms with which we are dealing is, therefore, worthy of note.

(5) The blood-pressure curve in Enhydrina poisoning is a remarkably steady one, provided that moderate doses are given and that care is taken to avoid the injection of large volumes of fluid into the blood-vessels. This is due to the fact that the

^{*} Paper communicated to the Royal Society, June 9, 1904.

blood pressure is exposed neither to the influence of the rival forces which act on the heart so strongly in cobraism, nor to the direct vaso-motor changes which we have dealt with in the preceding paragraph.

(6) The respiratory mechanism appears to be that which is chiefly affected by Enhydrina venom. If large lethal doses are employed, such as ROGERS appears to have confined himself to, respiration falls rapidly, and a considerable rise of blood pressure, asphyxial in origin, may precede death. The heart beat also quickly slows, and blood pressure falls with corresponding rapidity.

Obviously, these are simply the phenomena of rapid asphyxiation. If, however, smaller doses of venom are employed, no marked rise in blood pressure occurs. The ordinary level is maintained till near the occurrence of death; the beat then slows and the blood pressure falls. Here we have an expression of gradual cardiac failure, brought about by slowly progressive asphyxiation. The absence in slow Enhydrina poisoning of the large asphyxial rises of pressure, which are so characteristic of the final stages of Cobra poisoning, is readily explained by the fact that Enhydrina venom has no direct constrictive action on the walls of the arterioles, such as Cobra venom possesses.

(7) As to the part of the respiratory mechanism that is affected by Sea-snake venom, the rapidity with which respiration is modified, both when venom is injected into a vein and also when it is applied directly to the medulla oblongata, leaves no room to doubt that the respiratory centre is directly acted on by the venom. On the other hand, we have shown that some degree of motor nerve-end paresis is constantly present in animals dying from the effects of subcutaneous injections of this venom. We have also emphasised the fact that in our experiments carried out by dropping venom on the exposed medulla oblongata, we have failed to kill animals through the respiratory centre with the motor nerve-ends still undamaged. In this respect we have shown that Enhydrina venom differs in its action from Cobra venom. It would, therefore, appear that in poisoning with Enhydrina venom, motor nerve-end paresis plays a much greater part than it does in cobraism. It is not difficult to suppose that a blunting of the motor nerve-end mechanism, even though far from absolute, may seriously add to the embarrassment of a centre which has already been directly and gravely damaged. We hope to return to this and other points in a future communication.

In conclusion, we desire to express our sense of indebtedness to the India Office, to the Government of India, and last, but by no means least, to the Madras Government for the assistance and facilities which they have given us in the carrying out of this research.

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IX. *On the Action of Wood on a Photographic Plate in the Dark.*By WILLIAM J. RUSSELL, *Ph.D., F.R.S.*

Received May 28,—Read June 16, 1904.

[PLATES 11–18.]

It has been shown in former papers that if wood, even if old and dry, be placed on a photographic plate in the dark, it is able to act on it in such a way that when the plate is treated with an ordinary developing solution a clear picture of the structure of the wood is produced. Further experiments on this and allied subjects have been made, and interesting results obtained.

After experimenting with a large number of woods the conclusion is that, almost without exception, they have this property of acting on a photographic plate in the dark. The amount of action exerted, however, varies very considerably with different woods, some requiring a much longer time to produce a good picture than others. The ordinary limits of time and temperature are from $\frac{1}{2}$ –18 hours' exposure and from ordinary temperature to about 55° C. The time of exposure may be extended to 30 or 48 hours, but a higher temperature cannot be used, since plates, as a rule, are damaged when heated above 55° C. This action occurs both when the wood is placed in contact with the plate and when it is supported above the plate.

Some of the most marked and interesting results are obtained by woods belonging to the group of conifers. To take first the common Scotch fir (*Pinus sylvestris*). A transverse section of a branch or stem about 3 inches in diameter is a convenient size to use, as it will stand on a quarter-size photographic plate. The specimen used should be turned quite flat on one side and then rubbed with fine sand-paper till quite smooth. It is also necessary that the section be dry, or else on warming, water will deposit on the photographic plate and the picture be spoilt. Plate 11, fig. 1, shows the picture produced by Scotch fir treated as above described. What is active in the wood is dark in the picture. The whole picture is perfectly clear and sharp, so much so that it will bear considerable enlargement. On carefully examining this picture, and comparing it with the original wood, it will be found that it is the light-coloured rings, the spring wood, which have acted on the plate, and that the dark rings, the autumn growth, are entirely without action. As previously pointed out, this picture is very sharply defined and is quite constant; all specimens of this and allied pines act in exactly the same way.

It has previously been shown that probably hydrogen peroxide is the active agent

in producing pictures on a photographic plate in the dark, consequently it seems probable that the resin in the wood is indirectly the cause of the picture. That it is not a case of radio-activity appears to be proved, for a glass or mica screen of one-thousandth of an inch in thickness entirely protects the plate from being acted on. This great difference between the action of the spring and autumn rings in the Scotch and other pines seems at first difficult to explain, but the following facts throw some light on the matter and may help to an explanation. It is difficult to imagine that in the soft porous white wood there should be sufficient resinous matter to make it active, and at the same time resin should be entirely absent from the dark rings, yet that is what the photographs indicate. It is, however, not difficult to show that resin is contained in the dark rings, for if the material forming these rings be carefully cut out so as to separate them entirely from the white wood, and then treated with hot alcohol, the filtered solution on evaporation leaves a residue of resinous matter, which acts strongly on a photographic plate. Again, if this dark wood, which is devoid of action on a plate in its normal state, is reduced to a state of fine powder by scraping or rasping it, it then becomes very active, readily acting on a photographic plate; thus it would seem that in the autumn wood the resin is confined in cells through the walls of which it cannot pass. Even a moderate amount of heat does not change the action of this white and dark wood of the Scotch fir, for if separate specimens of the rings be heated to 90° or even to 120° C. for some hours, and after cooling be placed on a photographic plate for 18 hours, the light wood still gives a good dark picture and the dark wood only a very faint one.

That the amount of moisture in the wood does not affect these results was proved by drying the wood at 55° and cooling it in a desiccator over sulphuric acid, when the action exerted on the plate was found not to be altered. When even a little inactive oil or wax was rubbed on to a piece of deal it did not affect its activity. Fig. 2 is a picture of a tangential section of deal; other members of the pine group—namely, *P. pinaster*, *P. strobus*, and pitch pine—have been experimented with. Fig. 3 gives a picture of radial transverse and tangential sections of this latter fir.

With the spruce firs (*Piceæ*) the action on the plate is not of so definite a character as it is with the pines, for the dark rings are often active, the white wood, however, still remaining active, fig. 4.

The action of larch, which is also a member of the fir tribe, is very interesting, for it is the reverse of that of the Scotch fir, the dark rings being much more active than the spring wood. Fig. 5 is a picture of a transverse, and fig. 6 a tangential section of larch. The white wood in the larch is much harder than that in the Scotch fir, and, taken as a whole, the larch is a less active wood than the Scotch fir. In the cedar, which is so nearly allied to the larch, the dark rings appear to be the most active, but are so near together in the specimens examined that it is difficult to trace exactly their action. With the silver firs (*Abies*), the dark rings as in above cases are active. The *Cupressus* tribe all seem to give an active wood

and one in which the dark rings exercise more action on the plate than the light wood, fig. 7. The juniper gives a wood which is only very slightly active.

Passing on now to woods not belonging to the group of Conifers. The oak, fig. 8, is an active wood, also the beech, fig. 9, and the acacia (*Robinia*), fig. 10, and Spanish chestnut, fig. 11. Sycamore is also a very active wood.

The English woods which have been examined may be roughly arranged in three Groups, those which are very active, active, and slightly active.

Very active.		Active.		Slightly active.	
Oak.	Beech.	Maple.	Alder.	Laburnum.	
Sycamore.	Box.	Robinia.	Ash.	Lime.	
Scotch fir.	Cedar.	Rhododendron.	Currant.	Laurel.	
	Cherry.	Silver birch.	Elm.	Pear.	
	Cupressus.	Spanish chestnut.	Gorse.	Plane.	
	Hornbeam.	Spruce.	Horse chestnut.	Tulip tree.	
	Holly.	Walnut.	Ivy.	Yew.	
	Larch.		Juniper.		

Elm is a wood which is remarkably inactive, as is shown by fig. 12. The wood of the tulip tree is of the same character. It is very slightly active except round the edge, where it is very active, fig. 13. Different specimens of the same wood naturally vary considerably in activity.

Foreign woods.—Many of these are very active, but as the annual rings are not generally well defined, the pictures which they give are of a somewhat different character from those already described. The following is a rough list of the woods which have been tried, arranged as before in three groups :—

Very active.		Active.		Slightly active.	
African blackwood.	King wood.	Bass.	Hickory.	Partridge.	Sandal wood.
Bar wood.	Rose wood.	Bamboo.	Lignum vitæ.	Quebracho.	
Cocobolo.	Red saunders.	Cocos.	Mahogany.	Red gum.	
Kabucalli.	Snake wood.	Ebony.	Olive.	Teak.	
				Tulip wood.	

The following figures show some of the curious pictures which are produced by certain of these woods. Fig. 14 is produced by rose wood, fig. 15 by cocobolo, where the dark rings are inactive and the red ones are active. Fig. 16, lignum vitæ, transverse section, the outside yellowish ring is perfectly inactive and consequently not shown. Fig. 17 is bamboo, all the inner portion is perfectly inactive. In many of the foreign woods there is towards the outside a band of light-coloured wood which is inactive.

Knots in a wood are generally surrounded by resin, and it would therefore be naturally supposed that a very dense picture of a knot and its surroundings would

be formed, but this is not always the case. Fig. 18 shows a knot in yellow pine, and although the knot itself is dark its immediate surrounding, which is resin, is almost without action. In fact, resins evidently vary very considerably in their power of acting on a photographic plate, and their different degrees of volatility or general activity may be shown by placing the sensitive plate at a distance above the specimen of the wood and noting the result; for instance, a plate $\frac{3}{4}$ inch above a piece of deal will give a dark picture, whereas larch, pitch pine, or oak under similar conditions act only very slightly on the plate. Of course, the absolute amount of resin in each case is not known and varies considerably, but this variation in quantity does not seem to be the sole cause of the different effects produced. There is another way in which the resins from different sources may be tested. It is by placing the specimen of wood on a piece of Swedish filter paper, which is a perfectly inactive body, and heating it to a temperature of 90–100° C. for 2 hours, then when cold the paper is removed from the wood and is put up with a photographic plate. If larch, spruce, or red saunders wood has been used, then the paper on which it stood gives a distinct picture, and this is the case notwithstanding there being no visible sign of anything on the paper. Scotch fir, deal, beech, and oak will not act in this way, so that the larch at 90–100° is much more active when tested in this way than the Scotch fir.

It was evidently of interest, in place of a wood, to try the action of the ordinary commercial resin (*colophony*), and it was found to be very active, readily producing a dark picture. It can be used as a coarse powder, or by painting an alcoholic solution of it on one side of a piece of Bristol board and allowing this to dry and harden. Such a card will, if, for instance, different qualities of paper be laid upon it, give on the photographic plate very clear pictures, showing the structure and watermark of the papers. The resinous matter which is extracted by hot alcohol from different woods, as one would anticipate, varies much in properties. Deal and Scotch fir yield an oily residue which does not become hard, but is an active substance, whereas *lignum vitæ* and yellow pine give a resin which is also active and becomes hard. Larch, on the contrary, gives a hard resin which is not active or only very slightly so.

The following bodies, which range from resins to pure gums, are placed approximately in the order of their activity, ordinary resin being the most active on the list and the gums Senegal and Arabic being quite inactive:—

Strongly active.	Slightly active.	Not active.
Ordinary resin.	Dragon's blood.	Gum Senegal.
Burgundy pitch.	Asphaltum.	Gum Arabic.
Gum mastic.	Orange shellac.	
Gum damar.	Gum tragacanth.	
Venice turpentine.		
Gum copal.		

If a wood be wetted with ether, or even exposed to its vapour, it becomes more active, but the picture is less sharp. Merely heating a wood to 100° C. for 1 or 2 hours and then allowing it to cool does not alter its activity. If the heating be continued for, say, 8 hours, its activity is somewhat increased, but if continued for 100 hours the activity is considerably lessened.

To remove the whole of the resin from a wood so as to make it inactive is difficult. If it be digested with alcohol and ether, or with benzene, it retains its activity with great pertinacity, but if a shaving be plunged into boiling water for an hour it becomes perfectly inactive; also if it be treated with caustic alkali or gaseous hydrochloric acid or with chlorine it becomes inactive, but under less drastic treatment wood retains this power of acting on a photographic plate with great pertinacity. A piece of wood which had been exposed for many years on the roof of a London house was still active, so was an oak box more than 100 years old, and even decayed wood quite soft, taken from the stump of an old tree, and the central part of a piece of oak taken from a Dartmoor bog.

Generally the picture produced on the plate represents the structure of the wood with which it has been in contact and every part of the picture can be identified; but this is not always the case, for with certain woods a picture is always formed which is not visible to the eye even if the wood be examined with a magnifying glass. This is specially the case with bass wood. Fig. 19 is an ordinary photographic picture of a specimen of this wood and fig. 20 is the picture produced in the dark. Figs. 21 and 22 show the strong difference there is in the pictures of olive wood obtained by these different processes, and figs. 21A and 22A show the difference in cherry wood. With some specimens of the wood of *Abies pectinata* the same thing occurs. Bar wood also gives in the dark a picture in which apparently a large number of cracks are represented; these are not visible in the wood. That the picture produced in the dark is perfectly definite and constant is shown by the fact that if successive sections of the same branch be used the same picture is always produced. The botanist may be able to throw some light on this curious fact.

The true bark, the outside layer of a tree, appears to be in almost all cases quite devoid of activity, but the layer within it, often of a brownish colour, is very active, even more so than any other part of the wood. Fig. 23 is a transverse section of a small *Pinus pinaster*, showing that the outer layers, which were remarkably thick in this case, were more active than the wood itself. Fig. 24 represents two pieces of larch bark, and shows the great activity of the inner layer. With the oak and the beech the same thing holds good, the outside inactive and a thin layer between the bark and the wood very active. In the case of the *Rhizophora mucronata* wood the bark is about $\frac{1}{2}$ inch thick, but it acts in the same way as the other woods. In the cases where the resin canals come up within the bark they, of course, are very active. This is shown in fig. 25, where a series of dots are seen round the outside of the picture; these are the ends of the resin ducts, in a layer of bark, which is itself entirely inactive.

Ivy acts in a way different from most other woods, in having its outside layer active and the central part inactive; this is shown in fig. 26. This occurs in all cases where the central pith is largely developed. Fig. 27 is a section of a sunflower stem. The bamboo also gives a similar picture, its outer layer only being active.

In the case of different nuts it seems that the outside shell is very active, but not the kernel. Fig. 28 shows the picture produced by a section of a coco-nut.

There is another and very interesting action which occurs with wood, it is the action which light has upon it. The activity—that is, the power which wood has of acting on a photographic plate in the dark—is always increased by increasing the amount of light falling upon it. For instance, if two similar pieces of Scotch fir be taken, and one remain in ordinary light and the other is exposed to sunlight for 10 minutes, and then both be put up with photographic plates, the pictures they give will be of very different density. Or a still more conclusive experiment is to take one piece of wood and cover half of it with black paper or tinfoil, and expose to a bright light, then two pictures will be obtained from the same piece of wood which vary much in density. Fig. 29 is a picture of a piece of deal which was treated in this way.

Similar results were obtained with other woods; for instance, elm is one of the least active of woods, but after even a short exposure to sunlight it becomes very active, fig. 30. Fig. 31 shows the picture of ivy under ordinary conditions and after exposure to sunlight. Again, fig. 32 is a picture of a piece of teak, half of which has been exposed to sunlight. Even specimens of bark may, in this way, be stimulated into activity. Fig. 33 shows the pictures, on the same plate, of larch and oak bark in their ordinary condition and after a short exposure to sunlight. Even pith, which under ordinary conditions is practically inactive, becomes very active after exposure to sunlight.

It is important to note that this action of light on wood is not a general or all over action; it does not obliterate at once the picture which would naturally be formed, but the first action is simply to intensify this picture, and with stronger light the action increases and the picture becomes more and more indistinct. It is not necessary that the exposed wood should be in contact with the plate to obtain the increased effect, but if it be at some distance above it the action still takes place. This increased action of light, like the normal action of the wood, is entirely unable to pass through glass or mica.

This increase of activity produced by light does not immediately pass away. If the increase has been considerable the wood may retain some of its added energy for nearly a month, but in all cases the wood gradually returns to its former condition.

A piece of deal, after exposure to the sun for 9 hours and then kept in the dark for a month, had returned to its original state of activity. Two other pieces of deal were also exposed for 9 hours to sunlight, and then one was at once put

up with a plate, and the other, after being kept in the dark for 24 hours. This latter one gave a considerably lighter picture than the former one. It is not necessary to have direct sunlight to produce these effects. Two similar pieces of wood, one left in the middle of a room and the other placed at the window, when there was no sunshine, for about a couple of hours, gave a marked difference in their action on a photographic plate.

Artificial light acts on wood in the same way as sunlight does. A wood exposed to an arc light for one hour gives a good dark picture. also if 8 feet of magnesium ribbon be burnt at a distance of 3 inches from a wood it will cause a very considerable increase in its activity.*

It was interesting to ascertain whether a wood which had never been exposed to direct light would be capable of acting on a photographic plate, and to test this thick blocks of larch, oak, and deal were taken and sawn up in perfect darkness and a section from the central part of each put up with a plate without allowing it to come into the light. Each gave a good picture. Another piece of wood cut from the centre of a block and not exposed to light was kept for two months in the dark, but it still gave a picture of the same density as the former ones.

The action of light on the Spanish chestnut is remarkable, for it appears readily to stimulate the central portion of the section, but does not stimulate the outer portion. This is seen in fig. 34; the ring shows the outside of the specimen.

Resin is acted on by light in the same way as wood is. A simple way of proving this is to take, for instance, a 15-per-cent. alcoholic solution of ordinary resin and paint it on a piece of inactive Bristol board, and allow the resin surface to become perfectly dry and hard, then on exposure to sunlight it becomes very active. This is well shown in fig. 35, where a Japanese screen was placed on a resin card and exposed to sunshine for 3 hours. So great is the difference between the activity of the exposed and the unexposed resin that a sharp picture was obtained. Fig. 36 shows the effect produced on a gum guaiacum card, half of which was exposed to sunlight for 6 hours and the other half covered by a screen.

Ordinary cork is also acted on in the same way. An old card which had been varnished with picture copal and become only slightly active had its activity restored by exposure to sun light.

In a former communication it has been shown that printer's ink is able to produce a picture on a photographic plate; its activity is much increased by sunlight, and even old printing, which is now only slightly active, becomes much more so after exposure to sunlight. Leather and pure indiarubber, also fur and feathers have their activity increased by exposure in the same way, and it was interesting to find that paper which had been sized with resin on being exposed to sunlight had its activity much increased, whereas paper which had been sized with gelatine was not affected.

* The light from the electric discharge of a Ruhmkorff coil, with aluminium poles, also increases the activity of wood, but not to any great extent. Glass entirely prevents this action. July, 1904.

Not only are resins acted on by light, as already described, but so is turpentine and its allies. Two samples of turpentine placed close together in similar glass vessels, one being covered with a glass plate and the other with a metal one, were exposed to bright light for some hours; then, in the dark, each vessel was covered with a photographic plate, which was left on for 21 hours. The turpentine which had been under the glass plate gave by far the darkest picture.

With bodies such as metals, slate, porcelain, flour, sugar, in which there is no resinous or allied body, this action of light does not occur; and with pith, if it were perfectly pure, it would probably, after exposure to light, be absent. Having then proved that bright light had so strong an effect on woods and certain other bodies, the next step was to ascertain which rays were the most active in producing this effect. With this object strips of different coloured glasses were laid on sections of different woods and then exposed to sunlight.

The following figures show well the actions which took place. Fig. 37 is the picture of a piece of deal on which a strip of white glass and a strip of red glass were placed. The rest of the wood was left uncovered. Fig. 38 shows the action of a blue and a green glass and no glass on the wood, and fig. 39 shows how completely the effect of the sunlight is cut off by either a strip of black paper or tinfoil, although not more effectually than it is by a red glass. In the above case the exposure to sunlight was for 5 hours. Many different woods were tried, but the effects produced in all cases were of precisely the same character.

In place of using coloured glasses experiments were made with light which had been filtered through different coloured solutions. With this object wood specimens were placed in double-cased bell-jars which had either potassium bichromate, copper ammonium sulphate, or pure water in the double casing. Similar pieces of deal were placed in each bell-jar and exposed for 4 hours to sunlight. The deal specimen in the red light gave only a faint picture, the one in the blue a dark picture, and the one with the water alone a picture a little darker than the one in the blue light. Other woods acted in the same way, so did cards painted with resin, with guaiacum, and with copal varnish. The varnish obviously must not be very active or the action of the light is masked. White oil paint is also rendered very active by the blue rays, and the same applies to the resin-sized paper.

Another series of similar experiments were made by interposing in the light from an electric arc different coloured glasses and allowing the coloured light to fall on wood and also on other bodies; it was found that when a beam from the lamp which has passed through a red glass was allowed for $1\frac{1}{2}$ hours to fall on a wood section, it produced no effect, but when a blue glass was interposed and the beam fell on the wood section for only 1 hour a dark picture was produced. Other bodies, such as cork and resin paper, are affected by the coloured beams in the same way. A wood section which had been stimulated by sunlight on being kept in red light loses

its activity as it would do if kept in the dark. A spectrum formed with the arc lamp and thrown for $4\frac{1}{2}$ hours on a wood section produced no visible effect.

It has already been stated that this action of wood, even when stimulated by light, does not pass through glass, but it does through porous bodies and through gelatine; it can also be shown that it will pass through a certain thickness of wood. A piece of deal 1.4 millims. thick had a red glass and a green glass laid upon it and was exposed at a window for 3 days, then it was turned over and the photographic plate placed on the side which had not been exposed to the light; the picture it gave showed that underneath where the glasses had been there was very little action, but elsewhere there was much action. Both turpentine and pure terebene are acted on by blue light so as to increase greatly their action on a photographic plate. Some experiments with these liquids were made by suspending some Ford blotting paper in a bottle with the liquid covering the bottom of it till they had taken up some quantity of the vapour; in one set of experiments the bottle with the paper in it was placed in the bell jar with the coloured liquid, and in the other experiments the paper saturated with the vapour was taken out of the bottle and placed in the bell jar; in both cases the blue light produced a more active substance than the red light did. This is shown by fig. 40.

I desire to record my thanks to the managers of the Royal Institution for allowing me to carry out the foregoing work in the Davy-Faraday Laboratory. My best thanks are also due to my assistant, Mr. O. F. BLOCH, who has been of much help to me and has made all the illustrations for this paper.

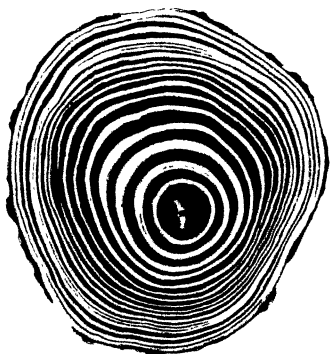


Fig. 1.—Scotch fir.



Fig. 2.—Deal.

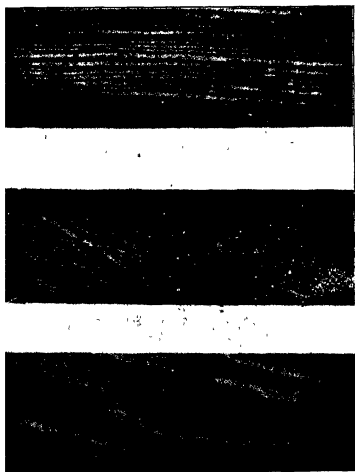


Fig. 3.—Pitch pine.

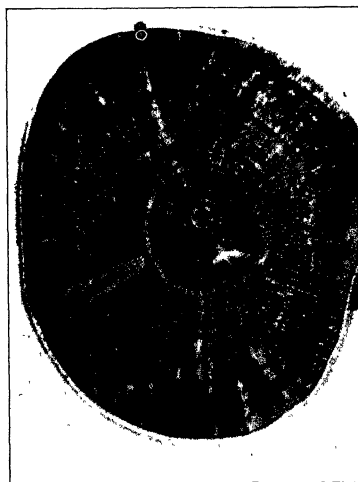


Fig. 4.—Spruce.



Fig. 5.—Larch.



Fig. 6.—Larch.

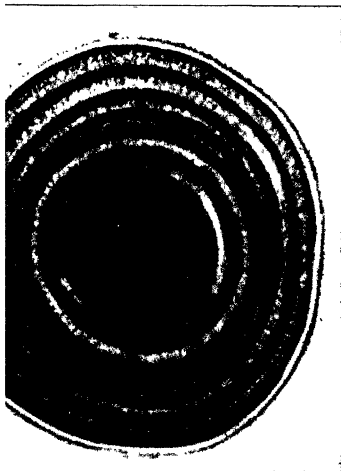


Fig. 7.—Cypress.

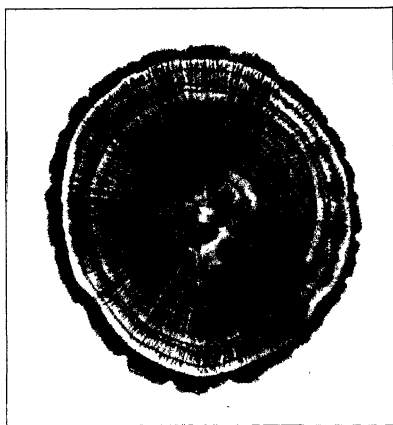


Fig. 8.—Oak.
(Three-quarters size.)

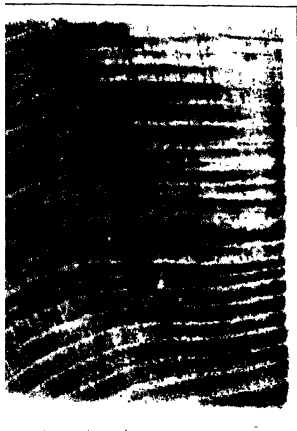


Fig. 9.—Beech.

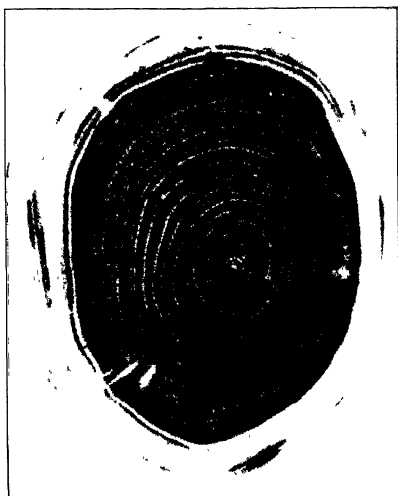


Fig. 10.—Robinia.
(Three-quarters size.)

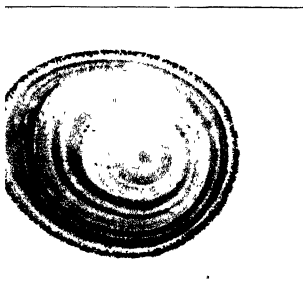


Fig. 13.—Tulip tree.

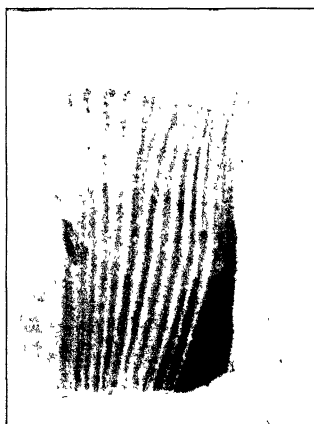
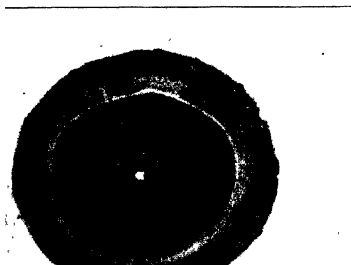




Fig. 14.—Rosewood.

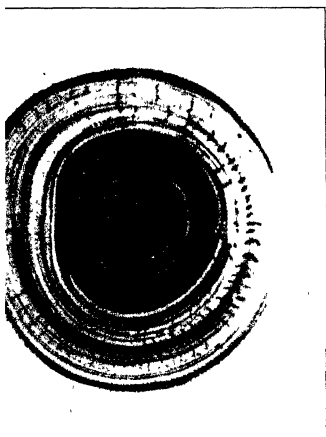


Fig. 16.—Lignum vitae.

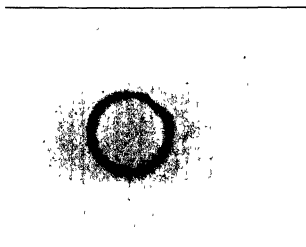


Fig. 15.—Cocobolo.

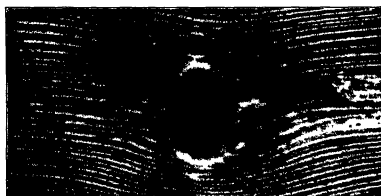


Fig. 18.—Yellow pine.



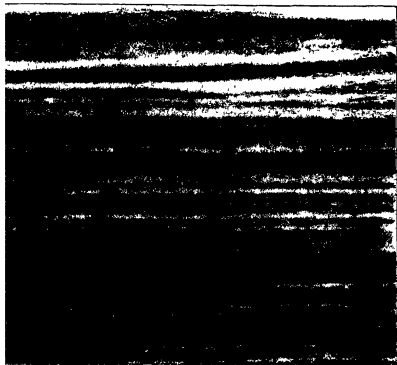


Fig. 20.—Basswood.



Fig. 21.—Olive.

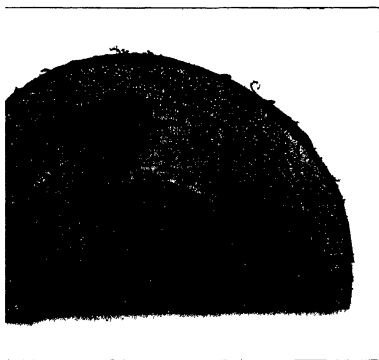


Fig. 21A.—Cherry.
(Three-quarters full size.)

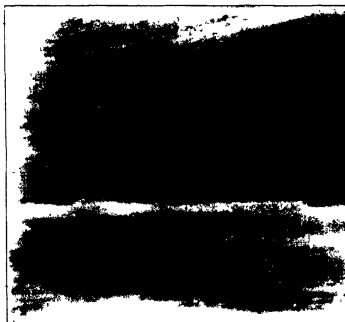


Fig. 22.—Olive.



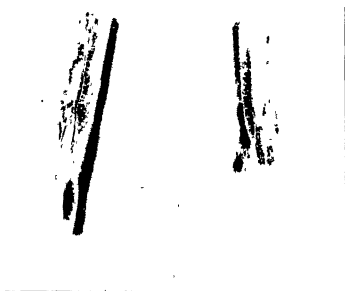


Fig. 24.—Larch bark.

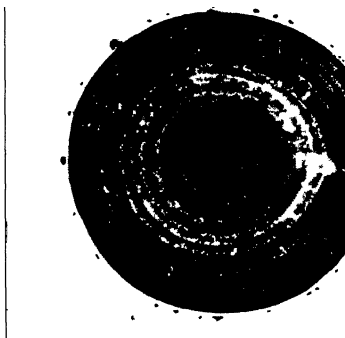
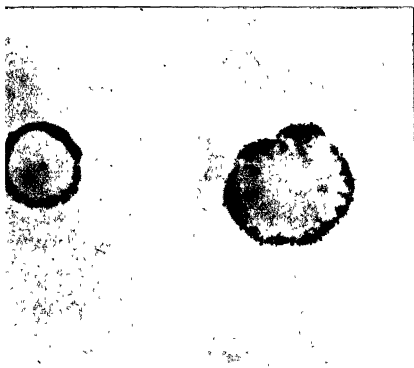


Fig. 25.—Rhizophora.



26.—Ivy.

Fig. 27.—Sunflower.

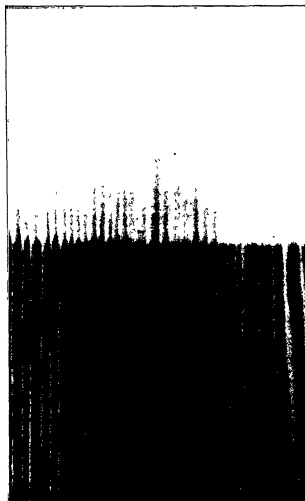


Fig. 29.—Deal.



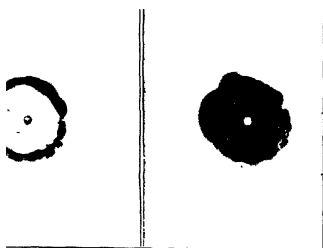
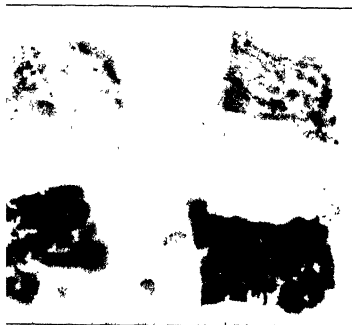


Fig. 31.—Ivy.



ch.

Fig. 33.

Oak.

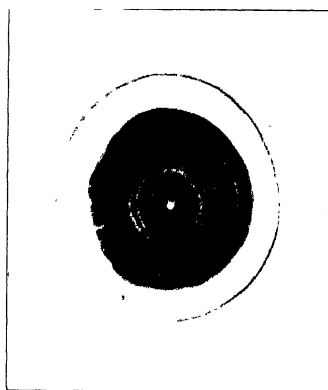
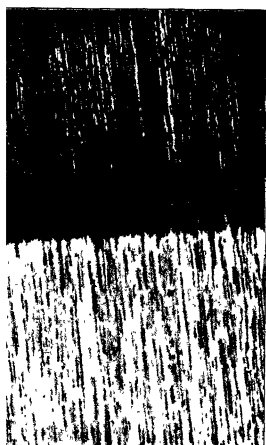


Fig. 34.—Spanish chestnut.

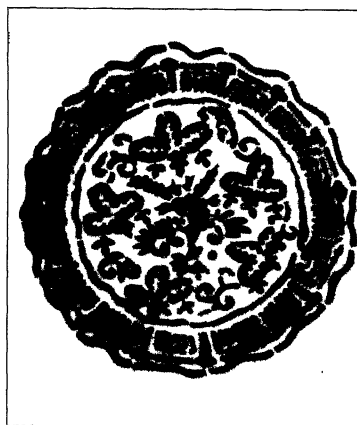
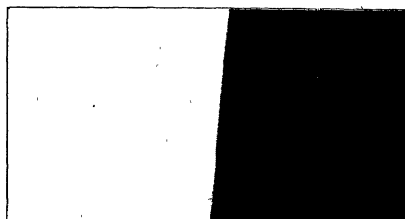
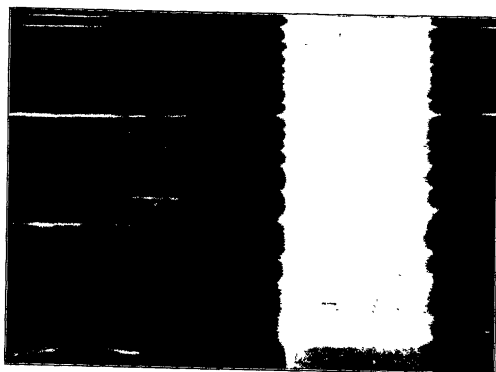


Fig. 35.—Japanese screen.





White.
Fig. 37.—Deal.

Red.



Green.
Fig. 38.—Deal.

Blue.



Fig. 40.



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Carbonicola from the Culm Measures in North-west Devon.

ARBRE, E. A. Newell. Phil. Trans., B, vol. 197, 1904, pp. 291-325.

Culm Measures of North-west Devon—Fossil Flora and Age.

ARBRE, E. A. Newell. Phil. Trans., B, vol. 197, 1904, pp. 291-325.

Devon (N.W.) Carboniferous Rocks—Fossil Flora and Age.

ARBRE, E. A. Newell. Phil. Trans., B, vol. 197, 1904, pp. 291-325.

X. The Fossil Flora of the Culm Measures of North-west Devon, and the Palaeobotanical Evidence with Regard to the Age of the Beds.

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Communicated by Professor T. MCKENNY HUGHES, F.R.S.

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[PLATES 19 AND 20.]

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1. *Introduction.*

THE deposits of Upper Palæozoic age in the south-west of England differ remarkably in their general characters from those developed on the north side of the Bristol Channel. This conclusion applies not only to the Devonian rocks, but also to the succeeding Carboniferous series. In South Wales and Monmouth, the Devonian beds are of the Old Red Sandstone type, whereas, the Devonian succession of North Devon exhibits a marked, though not an entire change, in both petrological characters and palæontological facies; a change which becomes even more marked in the series of limestones, volcanic, and detrital deposits developed in the southern portion of that county.

The South Wales coalfield, the largest and most important productive measures in

this country, consists of a sequence of coal-bearing strata, resting upon beds of Lower Carboniferous age, for the most part similar in character to those occurring in our other English coalfields. In Devonshire, and in portions of the neighbouring counties of Somerset and Cornwall, a Carboniferous basin of considerable size is developed, occupying more than 1,200 square miles. In many important respects these rocks again differ somewhat markedly from their equivalents in South Wales. They form a succession of deposits of a somewhat abnormal type; being composed of sediments of extremely varied nature and origin, both detrital and organic. They are especially characterised by a general absence of carbonaceous material of any economic importance. These Carboniferous rocks are spoken of as the Culm Measures, a name first applied to them by SEDGWICK and MURCHISON* in 1837. These authors† in their classic memoir, published in 1840, gave the first accurate description of the physical structure of the beds, and proved conclusively their Carboniferous age. It may be pointed out, however, that DE LA BECHE‡ in 1834, was the first to indicate the Upper Carboniferous age of that portion of the Culm Measures which forms the subject of this memoir; his conclusion being based on plant remains identified by Professor LINDLEY. DE LA BECHE§ also added considerably to our knowledge of the Culm Measures in his 'Report on the Geology of Cornwall and Devon,' published in 1839. Since then, JOHN PHILLIPS,|| HOLL,¶ T. M. HALL,** and others,†† and, in more recent times, Messrs. HINDE and FOX,‡‡ and Mr. USSHER,§§ have all contributed important information on this subject.

SEDGWICK and MURCHISON showed that the Culm Measures of Devon and the adjacent counties, occupy a true basin or syndinal fold between the Devonian succession in North and South Devon; the lower beds of the Culm Measures occupying the northern and southern margins. They instituted a two-fold subdivision of these beds, the Lower, and the Upper Culm Measures, and, although, in more recent times Mr. USSHER||| has urged the acceptance of a three-fold division, SEDGWICK and MURCHISON's classification is adopted here, for reasons which will be explained at a later stage in this work.

NOTE.—The numbers in parentheses after the authors' names denote the year of publication of the memoir, to which reference will be found in the bibliography at the end of this paper.

* SEDGWICK and MURCHISON (37), p. 557.

† SEDGWICK and MURCHISON (40).

‡ DE LA BECHE (34).

§ DE LA BECHE (39).

|| PHILLIPS (41).

¶ HOLL (68).

** HALL (75) and (78).

†† See bibliography at the end of this paper.

‡‡ HINDE and FOX (95).

§§ USSHER (92) and (01).

||| USSHER, *ibid.*

At the present time our knowledge of the Lower Culm Measures is on an altogether different footing to that of the Upper Division. This is almost entirely due to an important paper published in 1895, by Messrs. HINDE and FOX, in which the petrological characters and fauna of these beds were fully and admirably described. These authors showed that this division is of Lower Carboniferous age, and that it consists of Radiolarian cherts, overlying inconstant and impersistent limestones* in some areas, and dark shales; the latter apparently forming the basement beds. Above the Radiolarian rocks, commonly known as the Coddon Hill Cherts, lies the great series of sandstones and shales constituting the Upper Culm Measures, and forming by far the thickest portion of the Culm Measures in Devon. Owing, however, to the highly folded, contorted, and faulted nature of the strata, as the result of earth movements, it has not so far been possible to arrive at even an approximate estimate of the thickness of either of these divisions. It is, however, beyond dispute† that the Upper Culm Measures are of far greater vertical extent than the lower series.

These upper beds were not dealt with by Messrs. HINDE and FOX‡ in the paper referred to, although they expressed the opinion that "the question of their relative age can be determined only by their plant-remains."

The present paper is an attempt to add to our knowledge of the fossil flora of the Upper Culm Measures, more especially with a view to determining the horizons in the Upper Carboniferous which are represented in Devon. No attempt has been made here to unravel the extremely complicated geological structure of the district. Attention has, so far, been entirely devoted to the task of the collection of plant remains from the one horizon in the Upper Culm Measures in which carbonaceous deposits of appreciable thickness occur—a task rendered extremely difficult owing to the crushing and severe folding which the area has undergone in common with the rest of the Culm Measures.

The occurrence of an impure smutty coal, known locally as "culm" or "anthracite," is confined to a small area, for the most part in the neighbourhood of Bideford in North-west Devon. Such deposits are altogether absent in the Lower Culm Measures, and, with the exception of occasional carbonaceous partings in the shales and sandstones of the Upper Culm Measures of other districts, are not found elsewhere in either North or South Devon. This coal has in the past been largely worked in the Bideford district, the material being used in the manufacture of black paint, and also to some extent as a rough fuel. It is at present only mined at East-the-Water, Bideford.

The coal or "culm" is not only of local occurrence, but is practically limited to one horizon in the Upper Culm Measures. It forms a number of discontinuous and

* Mr. USSHER is inclined to regard the limestones as superior to the Coddon Hill beds. See USSHER (92), p. 121.

† See Mr. USSHER's recent map (01), Plate 16; also HINDE and FOX (95), p. 662.

‡ HINDE and FOX (95), p. 663.

inconstant bands of no great thickness, which range from the western coast at Greenacliff* in almost a straight line through Bideford for a distance of some 12 miles to the east, near Chittlehampton. The position of these bands is shown on the only Survey Map of North Devon so far published, that of DE LA BECHE, surveyed upwards of 60 years ago.† All the bands do not appear to lie on exactly the same horizon, several of them being parallel to one another, although they are all very closely associated. For the purposes which we are considering here, they may, however, be regarded as constituting one horizon, and are therefore of special value, for it has so far proved exceedingly difficult to recognise definite lithological horizons in the Upper Culm Measures, or to trace any particular beds from place to place, on account of the greatly disturbed nature of the rocks.

There is no reason to doubt that the horizon of the coal-bearing strata, near Bideford, lies nearer the base than the summit of the Upper Culm Measures, although, for reasons already explained, it is almost impossible to arrive at even an approximate idea of the thickness of the series until the district has been mapped in detail.

From a palaeobotanical standpoint the Culm Measures offer a most unpromising field. Although, as will be seen, a considerable number of species have already been described from the horizon of the culm or anthracite, it is nowadays a matter of the greatest difficulty to obtain specimens sufficiently well preserved to admit of satisfactory determination. All the plants described here have been obtained from the beds closely associated with the "culm," and are, therefore, practically from one horizon in the Upper Culm Measures.

I would wish to express here my obligations and thanks to several geologists who have given me useful information, or obtained for me the loan of specimens. I am especially indebted to Mr. INKERMANN ROGERS, of Bideford, who has generously placed his splendid collection of Culm Measure plants, gathered as the result of much patience and perseverance, at my disposal for examination and description. I am under great obligations to him, not only for the assistance he has rendered me during my visits to the Bideford district, but for the time which he has devoted, often at personal inconvenience, to the collection of specimens with a view to helping forward this work. To my friend, Mr. KIDSTON, F.R.S., I am indebted for assistance in difficulties of identification and for valuable advice. Mr. VIDAL, of Fremington, has rendered me great services by his local knowledge, and by guidance in the field. I am similarly indebted to Mr. HAMLING, F.G.S., of Barnstaple, especially for facilities for studying the specimens in the Hall Collection of Culm Measure plants at Barnstaple. I would also wish to express my thanks to the Director of the

* Also known as Greencliff.

† Mr. ROGERS, of Bideford, has kindly re-examined for me the direction of the several bands of culm or anthracite, and his conclusions agree in general with those of DE LA BECHE. For a description of the culm beds, see DE LA BECHE (39), p. 124 and p. 513, &c., HALL (75) and (78), and USSHER (92), p. 150.

Geological Survey for permission to examine the Culm Measure fossils in the Jermyn Street Museum, and to the Keeper of the Geological Department of the British Museum (Nat. Hist.) for access to similar specimens under his care. My sincere thanks are also due to Professor McKENNY HUGHES, F.R.S., on whose staff I have the honour to serve, for constant sympathy and encouragement in this and other palæobotanical work.

A.—THE FOSSIL FLORA OF THE UPPER CULM MEASURES.

II. *Previous Records of Fossil Plants.*

In almost every description of the rocks of the Upper Culm Measures in the neighbourhood of Bideford, mention is made of the common occurrence of plant remains. The name "floriferous slates" was, on this account, proposed for these beds by WILLIAMS in 1839. As a rule, however, such fossils are extremely badly preserved, and quite incapable of specific determination. A number of specimens have, however, been previously identified from North Devon, and these, so far as I am aware, were in all cases derived from the beds associated with the "anthracite or culm," or from the neighbouring grits and shales.

The earliest record of the discovery of specimens, sufficiently well preserved to admit of identification, is that of the collection formed by DE LA BECHE,* from the anthracite near Bideford. These plants were identified by LINDLEY, and his results were communicated to the Geological Society at the end of the year 1834.

LINDLEY'S Determinations.

Pecopteris lonchitica
Sphenopteris latifolia
Calamites canueformis‡
Asterophyllites resembling *A. longifolia*
A. sp., which may be *A. galioides*
Cypripites bicarinata§
Lepidophyllum intermedium.

Modern Names.†

Alethopteris lonchitica (SCHL.).
Mariopteris latifolia (BRONG.).
? C. suckowi, BRONG.
Calamocladus longifolius (SCHL.).
A. galioides (L. and H.).

Fragments, apparently of "Palm leaves"|| were also recorded, but the most abundant plant was stated to be too imperfect for determination.

In 1840, SEDGWICK and MURCHISON¶ added a further list of species from the

* DE LA BECHE (34), p. 106, footnote.

† Where no emendation is given, the original name still holds good.

‡ See KIDSTON (91), p. 364.

§ See KIDSTON (91), p. 359.

|| Probably crushed or distorted Calamitean pith-casts.

¶ SEDGWICK and MURCHISON (40), p. 681; also (37), p. 562.

shales overlying the culm bands about 2 miles to the east of Bideford.* These specimens were also determined by LINDLEY.

LINDLEY'S Determinations.		Modern Names.
<i>Asterophyllites foliosa</i> , L. and H.	=	<i>Annularia radiata</i> , BRONG.
<i>Calamites undulatus</i> , BRONG.		
<i>C. sp.</i> , resembling <i>C. arenaceus</i> , BRONG.	=	<i>C. sp.</i>
<i>Cyperites bicarinata</i> , L. and H.†		
<i>Neuropteris gigantea</i> , STERNB.		
<i>N. cordata</i> , BRONG.	=	<i>N. schuchzeri</i> , HOFFM.
<i>Pecopteris lonchitica</i> , BRONG.	=	<i>Alethopteris lonchitica</i> (SOHL.).
<i>P. muricata</i> , BRONG.	=	<i>Muriopteris muricalis</i> (BRONG.).
<i>Poaicetes cocoina</i> , L. and H.	=	<i>Calamites sp.</i>

Pecopteris lonchitica is stated to be "perhaps the *most abundant* of the *Bideford plants*."

DE LA BECHE‡ in his 'Report on the Geology of Cornwall, Devon, &c.,' mentions a number of species from the "range of carbonaceous rocks from Bideford Bay to Hawkridge Wood," which include several not previously recorded by him, or by SEDGWICK and MURCHISON. LINDLEY again determined these plants.

LINDLEY'S Determinations.		Modern Names.
<i>Pecopteris Serrii</i> , BRONG.	=	<i>Alethopteris serrii</i> (BRONG.).
<i>P. lonchitica</i> , BRONG.	=	<i>Alethopteris lonchitica</i> (SOHL.).
<i>Neuropteris Loshii</i> , BRONG.	=	<i>N. heterophylla</i> , BRONG.
<i>N. heterophylla</i> , BRONG.		
<i>Sphenopteris acuta</i> , BRONG.		

"Leaves of monocotyledonous plants§ of three or four species hitherto undescribed," and "probably also *Calamites nodosus*,|| and one which somewhat resembles *C. Steinhauri*," were also found, as well as "several species, unknown to Dr. LINDLEY, of the genera *Pecopteris*, *Asterophyllites*, *Sphenophyllum*, *Lepidodendron*, and *Poaicetes*.¶

MURCHISON** in his 'Silurian System' gives a list of eight species all of which were previously mentioned in his conjoint paper with SEDGWICK. In PHILLIPS†† 'Palæozoic fossils of Cornwall, Devon, &c.,' no record or figure is given of any plant remains from the Culm Measures.

* Possibly from Webbery Wood, see p. 299.

† See KIDSTON (91), p. 359.

‡ DE LA BECHE (39), p. 126.

§ Probably imperfect Calamitean casts.

|| *Calamites sp.* This is not a good species.

¶ Probably imperfect Calamitean casts.

** MURCHISON (39), p. 85.

†† PHILLIPS (41).

It may be also noticed that, in 1842, AUSTEN* recorded *Pecopteris lonchitica*, *Neuropteris heterophylla*, *Sphenopteris latifolia* or *acutifolia*, *Cyclopteris* sp. and *Calamites* from the Culm Measures of South-east Devon.

More than thirty years later, the late TOWNSHEND HALL,† a local geologist, gave a list of twenty-six Culm Measure species, which included all the determinations which had been published previously.

Modern Names.

<i>Asterophyllites foliosus</i> .	<i>Annularia radiata</i> , BRONG.
<i>A. galioides</i>	<i>Annularia galioides</i> (L. and H.).
<i>A. longifolia</i>	<i>Calamocladus longifolius</i> (SCHL.).
<i>Bowmanites</i> .	
<i>Calamites arenaceus</i> ?	<i>Calamites</i> sp.
<i>C. cannaeformis</i>	<i>Calamites</i> sp. †
<i>C. nodosus</i> ?	<i>Calamites ramosus</i> , ART. ‡
<i>C. Steinhaurii</i> ?	<i>Calamites</i> sp. †
<i>C. undulatus</i> .	
<i>Cyperites bicarinata</i> .	
<i>Lepidodendron</i> .	
<i>Lepidophyllum intermedium</i> .	
<i>Neuropteris cordata</i>	<i>N. scheuchzeri</i> , HOFFM.
<i>N. gigantea</i> .	
<i>N. heterophylla</i> .	
<i>N. Loshii</i>	<i>N. heterophylla</i> , BRONG.
<i>Pecopteris lonchitica</i>	<i>Alethopteris lonchitica</i> (SCHL.).
<i>P. muricata</i>	<i>Mariopteris muricata</i> (BRONG.).
<i>P. Serlii</i>	<i>Alethopteris serlii</i> (BRONG.).
<i>Poaicites coccoina</i>	<i>Calamites</i> sp.
<i>Sigillaria</i> .	
<i>Sphenopteris acuta</i> .	
<i>S. latifolia</i>	<i>Mariopteris latifolia</i> (BRONG.).
<i>Sphenophyllum</i> sp. ?	
<i>Sternbergia</i>	<i>Corluites</i> sp.
<i>Stigmaria ficoides</i> .	

In 1878 the same author§ gave a very similar list, and, so far as I am aware, this is the latest record of plants which were undoubtedly obtained from the Culm Measures. HULL|| in the fourth edition of 'The Coal-fields of Great Britain,' says that twenty-three species, including one marine plant, have been obtained from the "Culm," but gives no further details.

* AUSTEN (42), p. 461.

† HALL (75), p. 375.

‡ These species have no definite specific characters. They are probably all Stylocalamitean casts, and some of them may possibly be identical with *C. suckowi*, BRONG.

§ HALL (78), p. 66.

|| HULL (81), p. 64.

In 1884, Mr. KIDSTON* determined a number of plant remains, which were sent to him as probably derived from the Culm Measures, but without record of localities or horizons. Among the nine plants which he identified were *Asterocaulumites scrobiculatus* (SCHL.), *Calamites Roemeri*, Göpp., *Lepidodendron Rhodounum*?, and a new species of *Sphenopteris*. Chiefly on the occurrence of these species he regarded this flora as having a "Calciferosus Sandstone facies." It has been already pointed out by Messrs. HINDE and FOX† that there is no doubt that, while some of the specimens determined by Mr. KIDSTON may have been obtained from the Culm Measures, the above-mentioned species were derived from the Upper Devonian rocks (Marwood or *Cucullea* beds) at Sloy Quarry, near Barnstaple. Some of these plants are in the Athenæum Museum at Barnstaple. These records must, therefore, be excluded from any consideration of the Culm Measure flora.

III. Localities from which the Specimens, described in this Paper, were obtained.

The best preserved specimens have been found to occur, as a rule, in the black, highly carbonaceous shales closely associated with the smutty coal or culm. Thin partings of such shales also occur here and there in the sandstones and grits at some distance from the culm bands, and fossil plants have occasionally been found in them.

At East-the-Water, Bideford, the culm is still worked in a "Paint Mine" belonging to Mr. POLLARD, but no well-preserved specimens have been obtained from this locality. In Broadstone Quarry, near to the present culm workings, an adit through one of the culm-bands has been made, for the purpose of draining the quarry, and from the *débris* removed, or from the carbonaceous shales *in situ*, the specimens of *Lepidodendron fusiforme* and *Neuropteris schlehani*, figured here, were collected, in association with *Alethopteris serli*, *Calamites*, *Sphenophyllum*, *Renaultria*, *Sigillaria*, *Stigmaria*, and *Cordaites*.

In the neighbourhood of the present "Paint Mine," traces of former culm pits occur at Moor Park, and it was no doubt from these workings that the fine and interesting specimen of *Alethopteris lonchitica*, figured on Plate 20, fig. 15, was derived, which is now preserved in the Jermyn Street Museum.‡

Further to the east, at a spot some 500 yards to the north-west of Warmingtoun Farm, a culm-band crops out, and Mr. ROGERS has here obtained specimens of *Alethopteris*, *Mariopteris*, and *Stigmaria*.

There is a specimen in the Athenæum Museum, Barnstaple, of *Lepidodendron obovatum*, which was derived from the western limit of the culm, where it is exposed

* KIDSTON (84¹), p. 534, and (84²), p. 59.

† HINDE and FOX (95), p. 653.

‡ Registered number, 8423. Formerly in the collection of Lieut.-Colonel HARDING,

in the sea-cliff at Greenacliff. I have also collected *Sigillaria* from the same locality.

To the east of Bideford, a culm-band occurs near the village of *Alverdiscott*. Several specimens, collected by the late TOWNSHEND HALL, and now in the Athenæum, Barnstaple, are labelled with this locality. They include *Megalopteris* (?) *sp.*, and *Alethopteris lonchitica*, which are here figured, and also *Pinnularia*, the root of a *Calamite*.

Somewhat nearer to Bideford, numerous old culm workings may be found in Webbery Wood, and possibly this may have been the locality from which the species described by SEDGWICK and MURCHISON were obtained. I have not, however, been able to obtain further specimens from this culm band, as the former workings are now almost entirely obliterated.

The most important workings for culm, which have existed in past times, were probably those at Hiscott in the parish of Tawstock, at some distance to the east of Bideford, and to the south of Barnstaple. The culm-bands here were worked in the eighteenth century.* Mr. ROGERS has, on several occasions, carefully examined the *débris* around the old culm pits at Summer Hiscott, and has been successful in obtaining several well-preserved specimens from the dark carbonaceous shales. The examples figured here of *Alethopteris serli*, *Lepidophloios acerosus*, *Sigillaria tessellata*, and *Lepidodendron aculeatum* were collected from these beds. *Calamostachys longifolia*, *Calamocladus charaformis*, *Alethopteris lonchitica*, *Neuropteris obliqua*, *Sphenophyllum*, and *Mariopteris* also occur here.

The sandstones and shales on or near the horizon of the culm-bands have occasionally been found to yield well-preserved plant remains, though as a rule they are entirely barren, or the fossils are so badly preserved that specific identification is impossible. These rocks belong to the petrological facies termed by Mr. USSHER† the *Morchard type*, which he describes as follows: "Thickly and thinly bedded, greenish-grey and reddish, much jointed sandstones, associated with ovoidally splitting shales."

The sandstones have been long quarried near the village of Abbotsham, some 2 miles to the west of Bideford, at a locality known as Pit or ROGERMAN'S quarry. Several different workings have been made here at various times, and in some of them, partings of carbonaceous shale have been found which have yielded plant remains, such as *Annularia radiata*, *Sphenophyllum sp.*, *Calamites sp.*, and *Sigillaria sp.* Mr. ROGERS has been particularly fortunate in obtaining the well-preserved specimen of *Sigillaria scutellata*, figured on Plate 19, fig. 4, from the sandstones of the present quarry. The state of preservation is here almost unique as compared with other impressions from the grits. *Trigonocarpus parkinsoni* and *Stigmaria* were also found at Pit quarry.

* HALL (75), p. 369.

† USSHER (01), p. 362; see also (92), pp. 115 and 146.

The cliff above the railway station at Bideford is largely composed of thick shales, which have yielded numerous Calamites of various species, some of which are figured here. In a small sandstone quarry, belonging to the Rev. E. ROBERTS, of Bideford, at East-the-Water, and near the "paint mine" already mentioned, ovoidally splitting, highly ferruginous shales are seen. These have been carefully examined, and from them the figured specimens of *Urnatopteris tenella*, *Mariopteris muricata* were obtained, as well as numerous Calamites, and such types of Calamitean foliage as *Annularia radiata*, *A. galioides*, and *Calamocladus equisetiformis*.

There are very few specimens of fossil plants from the Culm Measures to be found in any museum. The British Museum (Natural History), possesses two collections, but few of the specimens are well preserved. One, presented by the Rev. W. BRITON in 1837, was chiefly obtained from Bideford and Northam. Many of the specimens* are, however, too poorly preserved to admit of specific determination. The other† formed part of the late TOWNSEND HALL's collection, purchased in 1886, nearly all of which were derived from Pit quarry, Abbotsham. The remainder of HALL's collection is in the museum of the Athenæum, Barnstaple. These specimens are described here, with the exception of a Calamite-like cast from Burrington, which I have been unable to determine.

The Jermyn Street Museum has the fine specimen of *Alethopteris lonchitica* already mentioned, and also a Calamitean-like cast (No. 8425), stated to be from Crediton. There are a few badly preserved Calamites from the Culm Measures in the museum of the Geological Society. I have not, however, been able to trace either SEDGWICK and MURCHISON's or DE LA BECHE's collections.

Most of the specimens described here were obtained during the past year specially for this memoir. The majority of them are from the fine collection of Mr. ROGERS, of Bideford, which it is hoped will eventually find a place in the British Museum. The specimens which I have collected during my visits to the Bideford district are now incorporated with the collections in the Sedgwick (Woodwardian) Museum, Cambridge (Carboniferous Plant collection, Nos. 1130-1156).

* Registered numbers in the Geological Department, 145-219.

† Registered numbers in the Geological Department, V 3561-74.

IV. *Description of Specimens.*

EQUISETALES.

Calamites. SUCKOW, 1784. ('Act. Acad. Elect. Theod.-Palat.,' vol. 5, p. 355).

1. *Calamites (Calamitina) undulatus*, STERNBERG. Plate 19, figs. 1 and 3.

1828. *Calamites undulatus*, BRONGNIART, 'Hist. végét. foss.,' p. 127, Plate 17, figs. 1-4.

1886. *Calamites undulatus*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 338, Plate 54, figs. 1 and 4.

Several specimens of *Calamites undulatus* have been obtained from the cliff above Bideford railway station. The two specimens from Mr. ROGERS's collection, figured here, show the flexuous character of the longitudinal ridges on the internodes. That on Plate 19, fig. 1, is a slightly enlarged photograph of a node, showing the characteristic termination of the ridges. The pith cast shown on fig. 3 of the same plate is reduced to half natural size. The flexuous ridges are here very clear.

2. *Calamites (Eucalamites) ramosus*, ARTIS. Plate 19, fig. 2.

1884. *Calamites (Eucalamites) ramosus*, WEISS, 'Steinkohl.-Calamarien,' Part 2, p. 98, Plate 2, fig. 3; Plate 5, figs. 1 and 2; Plate 6, figs. 1-7; Plate 7, figs. 1 and 2; Plate 8, figs. 1, 2, 4; Plate 9, figs. 1 and 2; Plate 10, fig. 1; Plate 20, figs. 1 and 2.

1887. *Calamites ramosus*, STUR., 'Die Calamarien Carbon.-Flora Schatz. Schicht.,' p. 96, Plate 12, figs. 1-4; Plate 12b, figs. 1-4 and 6; Plate 13, figs. 1-9; Plate 14, figs. 3-5, text-figs. 1 (p. 4), 31 (p. 104), 32 (p. 105).

Calamites ramosus is fairly abundant in the Culm Measures, and specimens have been obtained from the shales above the railway station, and in Mr. ROBERTS's quarry, Bideford, as well as from the present "paint mine." The specimen figured on Plate 19, fig. 2, is a fragment showing part of an internode, and a large branch scar at the node. In this species two branch scars of large size occur at every node. *Annularia radiata* is the foliage of this species.

3. *Calamites (Stylocalamites) suckowi*, BRONGNIART.

1828. *Calamites Suckowii*, BRONGNIART, 'Hist. végét. foss.,' p. 124, Plate 15, figs. 1-6; Plate 16, figs. 2-4.

1876-84. *Calamites Suckowi*, WEISS, 'Steinkohl.-Calamarien,' Part 1, p. 123, Plate 19, fig. 1; Part 2, p. 129, Plate 2, fig. 1; Plate 3, figs. 2, 3; Plate 4, fig. 1; Plate 27, fig. 3.

Calamites Suckowi is apparently not uncommon in the Culm Measures, specimens having been obtained from Broadstone, and ROBERTS's quarries at East-the-Water, Bideford. This species is one of the commonest in the Coal Measures.

4. *Calamites (Calamitina) varians*. STERNBERG.

1828. *Calamites dubius*, BRONGNIART, 'Hist. végét. foss.,' p. 130, Plate 18, figs. 1-3.

1884. *Calamites varians*, WEISS, 'Steinkohl.-Calamarien,' Part 2, p. 6, Plate 25, fig. 2; Plate 27, fig. 2; Plate 28, figs. 1-2 and 4.

The only specimens of *Calamites varians* from the Culm Measures which I have seen are two in the British Museum (Natural History), (V. 3563 and V. 3569), one of which was obtained by TOWNSHEND HALL from Pit quarry, Abbotsham.

Calamites sp. (External surface of the stem.)

MR. ROGERS has in his collection (No. 54) a small fragment of a Calamite obtained from Broadstone quarry, Bideford, which exhibits the external surface of the bark and some branch scars at the node. The specimen is too fragmentary to determine specifically. It is, however, interesting from the fact that such specimens are of rare occurrence in the British Coal Measures.

Annularia. STERNBERG, 1821. 'Flora der Vorwelt,' Heft 2, p. 32.

1. *Annularia radiata*. BRONGNIART.

1886. *Annularia radiata*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 394, Plate 59, fig. 8; Plate 61, figs. 1 and 2.

Annularia radiata has been collected by myself at ROBERTS's quarry, Bideford, and from Summer Hiscott by MR. ROGERS. There is also a specimen from Abbotsham in the Athenæum Museum, Barnstaple. This type of Calamitean foliage is now known to belong to *Calamites ramosus*.

2. *Annularia galioides*. (LINDLEY and HUTTON.)

1886. *Annularia microphylla*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 392, Plate 60, figs. 3 and 4.

1893. *Annularia galioides*, KIDSTON, 'Trans. Roy. Soc.,' Edinb., vol. 37, p. 317, Plate 2, figs. 4, 4A.

I have obtained a few specimens of *Annularia galioides* from ROBERTS's quarry, Bideford, but this species has not, I believe, been found elsewhere in the Culm Measures.

Calamocladus, SCHIMPER, 1869. ('*Traité Paléont. végét.*,' vol. 1, p. 323.)

1. *Calamocladus equisetiformis* (SCHLOTHEIM).

1836. *Hippurites longifolia*, LINDLEY and HUTTON, '*Foss. Flora*,' vol. 3, Plates 190 and 191.

1886. *Asterophyllites equisetiformis*, ZEILLER, '*Flore foss. bass. houill. Valenciennes*,' p. 368, Plate 58, figs. 1-7.

A single specimen of *Calamocladus equisetiformis* has been obtained from ROBERTS'S quarry, Bideford.

2. *Calamocladus charæformis*. (STERNBERG.)

1825. *Bechera charæformis*, STERNBERG, '*Flora der Vorwelt*,' Heft 4, p. xxx, Plate 55, fig. 3.

1887. *Asterophyllites Roehli*, STUR, '*Die Calamarien Carbon-Flora Schatz. Schicht*,' p. 209, Plate 14, figs. 13a, 13b, 13c; Plate 15b, fig. 3.

Several specimens of *Calamocladus charæformis* have been obtained by Mr. ROGERS from Summer Hiscott. Mr. KIDSTON, to whom I have submitted them, agrees with this determination. *Calamocladus charæformis* is now known to be identical with SAUVEUR'S *Annularia microphylla*.

Calamostachys, SCHIMPER, 1869. ('*Traité Paléont. végét.*,' vol. 1, p. 328.)

Calamostachys longifolia, WEISS.

1876. *Calamostachys longifolia*, WEISS, '*Steinkohl.-Calamarien*,' Part 1, p. 50, Plate 10, fig. 1.

A single specimen of *Calamostachys longifolia*, obtained from Summer Hiscott, has been determined by Mr. KIDSTON, who remarks that there seems to be some confusion with regard to this cone.

Pinnularia, LINDLEY and HUTTON, 1834. ('*Fossil Flora*,' vol. 2, p. 81.)

Pinnularia sp.

Specimens of the roots of a Calamite have been obtained from ROBERTS'S quarry, Bideford, and others from Alverdiscott are in the Barnstaple Athenæum. In these specimens, there seem to be several rows of rootlets springing from the main root, or possibly the rootlets are arranged spirally; the preservation not being sufficiently good to determine the exact arrangement. Similar fossils have been figured by ARTHUR in his '*Antediluvian Phytology*,' Plate 5, and by WEISS in the first part of his '*Steinkohlen Calamarien*,' Plate 30, fig. 1A. In other roots, included in the genus *Pinnularia*, the rootlets are arranged in two rows,

SPHENOPHYLLALES.

Sphenophyllum, BRONGNIART, 1822. ('Sur Class. végét. foss.,' p. 34.)

Sphenophyllum cuneifolium (STERNBERG).

1886. *Sphenophyllum cuneifolium*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 413, Plate 62, fig. 1; Plate 63, figs. 1-10.

1893. *Sphenophyllum cuneifolium*, ZEILLER, 'Mém. Soc. Géol. France Paléont.,' vol. 4, Mém. No. 11, p. 12, Plate 1, figs. 1-4; Plate 2, figs. 1-3; Plate 3, figs. 1 and 2.

Sphenophyllum cuneifolium is the only species which has been met with in the Culm Measures. It is fairly abundant, numerous examples having been obtained from Hiscott, from Broadstone quarry, at Bideford, and other localities.

CYCADOFILICES.

Neuropteris, BRONGNIART, 1822. ('Sur Class. végét. foss.,' p. 33.)

1. *Neuropteris obliqua* (BRONGNIART).

1832. *Pecopteris obliqua*, BRONGNIART, 'Hist. végét. foss.,' p. 320, Plate 96, figs. 1-4.

1886. *Neuropteris obliqua*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 284, Plate 48, figs. 1, 2, 4-7.

Neuropteris obliqua has been obtained from Summer Hiscott, and possibly also from ROBERTS's quarry, Bideford.

2. *Neuropteris schlehani*, STUR. Plate 20, figs. 10 and 13.

1877. *Neuropteris Schlehani*, STUR, 'Culm Flora,' pt. 2, p. 183, Plates 11 (28), figs. 7 and 8.

1886. *Neuropteris Schlehani*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 280, Plate 46, fig. 3; Plate 47, figs. 1 and 2.

Several specimens of *Neuropteris schlehani* have been obtained by Mr. ROGERS from the culm shale at Broadstone quarry, Bideford. Two of these are figured on Plate 20, figs. 10 and 13. Both these photographs are considerably enlarged (more than three times) to show the characteristic nervation. So far as I am aware, this constitutes the first British record of STUR's species, though Mr. KIDSTON, who has kindly identified the specimens figured here, has previously obtained specimens from the Middle Coal Measures. This frond is fairly abundant in some of the Continental coal-basins.

Alethopteris, STERNBERG, 1825. ('Flora der Vorwelt,' Heft. 4, p. xxi.)

1. *Alethopteris serli* (BRONGNIART). Plate 20, fig. 14.

1886. *Alethopteris Serli*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 234, Plate 36, figs. 1 and 2; Plate 37, figs. 1 and 2.

Alethopteris serli has been obtained by Mr. ROGERS both from Summer Hiscott, and from Broadstone quarry, Bideford. It appears, however, to be fairly rare in the Culm Measures on this horizon; at any rate it is very much less abundant than *A. lonchitica*. The enlarged photograph ($\times 3\frac{1}{2}$) on Plate 20, fig. 14, shows the characteristic habit and nervation of the pinnules; cf. *A. lonchitica*, figs. 11 and 15. *A. serli* is most abundant in the Upper Coal Measures, but is known to occur occasionally in the Middle Coal Measures of several British coalfields.

2. *Alethopteris lonchitica* (SCHLOTHEIM). Plate 20, figs. 11 and 15.

1828. *Pecopteris lonchitica*, BRONGNIART, 'Hist. végét. foss.,' p. 275, Plate 84, figs. 1-7.

1901. *Alethopteris lonchitica*, KIDSTON, 'Proc. Yorks. Geol. and Polytech. Soc.,' vol. 14, pp. 195, 219, Plate 32, figs. 2 and 3.

Alethopteris lonchitica is one of the most abundant of fern-like fronds in the Culm Measures, and has been found to occur in all the localities in which the culm-shales have been examined. The specimen figured on Plate 20, fig. 11, from Alverdiscott, shows the usual form of the pinna. Fig. 15 represents an apical portion of another pinna, in which the pinnules are not decurrent, but constricted basally, and apparently attached to the axes by very small portions of the somewhat cordate base. This variety is not rare in the Coal Measures, and is apparently very common in the Culm Measures. This specimen was obtained many years ago from Moor Park, near Bideford, and is now in the Jermyn Street Museum.

FILICALES?

Mariopteris, ZEILLER, 1879. ('Bull. Soc. Géol., France. Sér. 3, vol. 7, p. 92.)

Mariopteris muricata (SCHLOTHEIM). Plate 20, fig. 17.

1885. *Diplothmema muricatum*, STUR, 'Die Farne Carb.-Flora Schatz. Sicht.,' p. 393, Plate 21, figs. 1-5; Plate 23, figs. 1-6.

1901. *Mariopteris muricata*, KIDSTON, 'Proc. York. Geol. and Polytech. Soc.,' vol. 14, pp. 195 and 219, Plate 32, figs. 1, 1A.

Mariopteris muricata is a fairly abundant fern-like fossil in the Culm Measures in all the localities from which plants have been collected. The specimen

figured on Plate 20, fig. 17, was obtained from the ovoidally splitting shales at ROBERTS'S quarry, East-the-Water, Bideford, and is now in the Sedgwick Museum, Cambridge.

Urnatopteris, KIDSTON, 1884. ('Quart. Jour. Geol. Soc.,' vol. 40, p. 594.)

Urnatopteris tenella (BRONGNIART). Plate 19, fig. 6.

1828. *Sphenopteris tenella*, BRONGNIART, 'Hist. végét. foss.,' p. 186, Plate 49, fig. 1.

1882. *Eusphenopteris tenella*, KIDSTON, 'Ann. and Mag. Nat. Hist.,' Ser. 5, vol. 10, p. 7, Plate 1, figs. 1-6.

A solitary specimen of *Urnatopteris tenella* has been obtained from the shales exposed in Mr. ROBERTS'S quarry, East-the-Water, Bideford, and is now in Mr. ROGERS'S collection. A photograph of an enlarged drawing of this delicate frond is reproduced on Plate 19, fig. 6.

Renaultia, ZEILLER, 1883. ('Ann. Sci. Nat.,' Sér. 6, Bot., vol. 16, p. 185.)

1. *Renaultia footneri* (MARRAT).

1872. *Sphenopteris Footneri*, MARRAT, 'Proc. Liverpool Geol. Soc.,' p. 101, Plate 8, figs. 2, 3.

1890. *Sphenopteris Footneri*, KIDSTON, 'Trans. Roy. Soc.,' Edinb., vol. 35, p. 406, Plate 2, figs. 3, 3A, 3B.

Several specimens of *Renaultia footneri* have been obtained by Mr. ROGERS from Broadstone quarry, Bideford. Mr. KIDSTON, who has kindly identified this species for me, thinks that *R. footneri* may possibly be identical with *Sphenopteris gracilis* of BRONGNIART.* As, however, there would still seem to be some doubt on this question, it may be as well to maintain MARRAT'S species for the present.

2. *Renaultia schatzlarensis* (STUR.) ?

1885. *Calymmotheca schatzlarensis*, STUR, 'Die Farne Carb.-Flora Schatzl. Schicht.,' p. 265, Plate 38, figs. 1 and 2.

1888. *Crossotheca fimbriata*, KIDSTON, 'Ann. and Mag. Nat. Hist.,' Ser. 6, vol. 2, p. 23, Plate 1, figs. 1-3.

Mr. KIDSTON has somewhat doubtfully identified a specimen, obtained by Mr. ROGERS from Broadstone quarry, Bideford, as *Renaultia schatzlarensis*. It would be interesting if further examples of this plant could be obtained from the Culm Measures.

* KIDSTON (90), 4th Rep., p. 104.

Megalopteris, DAWSON, 1871. ('Foss. Plant. Dev. and Upper Sil. Canada,' Part 1, p. 51.)

Megalopteris sp.? Plate 20, fig. 16.

The specimen figured on Plate 20, fig. 16, which is in the museum of the Athenæum, Barnstaple, is labelled as having been derived from Alverdiscott, and the shale on which it occurs corresponds closely with undoubted Culm Measure shales from that locality. The specimen is unfortunately only a small fragment of what must once have been a frond of some size. Its most striking character is the great thickness of the mid-rib. The secondary nervation, arising from the mid-rib, is frequently dichotomised, and fairly close and oblique. In one or two places there appear to be anastomoses between the secondary nerves, but these are rare, and very possibly may be accounted for as accidents in the preservation.

This frond is quite unknown to me, but Mr. KIDSTON, to whom I have submitted the specimen, has suggested that it may perhaps be compared with DAWSON'S* genus *Megalopteris*, which is chiefly known from the Lower Coal Measures of Canada and the United States.† Unfortunately the specimen here figured will only permit me to assign it to this genus in a most guarded manner, as it is too fragmentary to indicate the real habit of the frond. So far as one can judge from the characters of the mid-rib and lateral nervation it agrees fairly well with *Megalopteris*. This genus has not so far been recorded from Britain. In some respects this plant also recalls the frond of *Glossopteris*, but it appears to me that the nervation is only very occasionally anastomosed, and that the union between the lateral veins is more apparent than real.

LYCOPODIALES.

Lepidodendron, STERNBERG, 1820. ('Flora der Vorwelt,' Heft 1, p. 23.)

1. *Lepidodendron aculeatum*, STERNBERG. Plate 19, fig. 8.

1820. *Lepidodendron aculeatum*, STERNBERG, 'Flora der Vorwelt,' Heft 1, pp. 20 and 23, Plate 6, fig. 2; Plate 8, fig. 1B, *a* and *b*.

1886. *Lepidodendron aculeatum*, ZEILLER, 'Flores foss. bass. houill. Valenciennes,' p. 435, Plate 65, figs. 1-7.

The photograph reproduced on Plate 19, fig. 8, is taken from the only specimen of *Lepidodendron aculeatum* which has been obtained from the Culm Measures. It was found among the *débris* of the old culm workings at Summer Hiscott by Mr. ROGERS. The leaf bases are well preserved, and the prints of the ligule, the leaf scars, and the ridges on the keel are clearly seen.

* DAWSON (73), p. 51, Plate 17, figs. 191-4.

† ANDREWS (75), p. 415, Plate 46, figs. 1 and 1a.

2. *Lepidodendron obovatum* STERNBERG.

1820. *Lepidodendron obovatum*, STERNBERG, 'Flora der Vorwelt,' Heft 1, pp. 20 and 23, Plate 6, fig. 1; Plate 8, fig. 1A, *a* and *b*.

1886. *Lepidodendron obovatum*, ZEILLER, 'Flora foss. bass. houill. Valenciennes,' p. 442, Plate 66, figs. 1-8.

The only specimen of this species which I have seen from the Culm Measures is in the HALL collection of the Athenæum Museum, Barnstaple. It was obtained from Greenacliff, on the coast some 3 miles to the west of Bideford.

3. *Lepidodendron fusiforme* (CORDA). Plate 19, fig. 5.

1903. *Lepidodendron fusiforme*, KIDSTON, 'Trans. Roy. Soc.,' Edinb., vol. 40, Part 4, p. 809, Plate 2, figs. 17 and 18; Plate 3, figs. 22-25.

The specimen of *Lepidodendron fusiforme* figured on Plate 19, fig. 5 was obtained from Broadstone quarry, Bideford, and is the only example known from the Culm Measures. This species, which has become involved in considerable confusion, has recently been defined and re-described by Mr. KIDSTON (see above), who has also published further figures of British specimens. It occurs in all the divisions of the British Coal Measures.

Lepidophloios, STERNBERG, 1825. ('Flora der Vorwelt,' Heft 4, p. xiii.)

Lepidophloios acerosus (L. and H.). Plate 19, fig. 9.

1831. *Lepidodendron acerosum*, LINDLEY and HUTTON, 'Fossil Flora,' vol. 1, Plate 7, fig. 1; Plate 8.

1893. *Lepidophloios acerosus*, KIDSTON, 'Trans. Roy. Soc.,' Edinb., vol. 37, Part 3, p. 558, Plate 1, figs. 1, 1a; Plate 2, fig. 9.

The photograph on Plate 19, fig. 9 represents a small fragment of *Lepidophloios acerosus* from the carbonaceous shales at Summer Hiscott. It is the only specimen of the genus which has been obtained from the Culm Measures.

Sigillaria, BRONGNIART, 1822. ('Sur Class. végét. foss.,' p. 9.)

1. *Sigillaria tessellata*, BRONGNIART. Plate 19, fig. 7.

1828. *Sigillaria tessellata*, BRONGNIART, 'Hist. végét. foss.,' p. 436, Plate 156, fig. 1; Plate 163, figs. 1-4.

1886. *Sigillaria tessellata*, ZEILLER, 'Flora foss. bass. houill. Valenciennes,' p. 561, Plate 85, figs. 1-9; Plate 86, figs. 1-6.

The photograph, reproduced on Plate 19, fig. 7, is taken from a fine specimen of *Sigillaria tessellata* obtained from the old culm workings at Summer Hiscott. This is the only example of this species which has, as yet, been found in the Culm Measures.

2. *Sigillaria scutellata*, BRONGNIART. Plate 19, fig. 4.

1828. *Sigillaria scutellata*, BRONGNIART, 'Hist. végét. foss.,' p. 455, Plate 150, figs. 2 and 3; Plate 163, fig. 3.

1886. *Sigillaria scutellata*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 533, Plate 82, figs. 1-6 and 9.

The large stem of *Sigillaria scutellata*, of which a portion is figured on Plate 19, fig. 4, was obtained by Mr. ROGERS from the sandstones at Pit quarry, Abbotsham, and is remarkably well preserved for a sandstone cast. The form of the leaf scar is clearly seen. The same species has also been obtained from the carbonaceous shales at Broadstone quarry, Bideford. Decorticated *Sigillarias* of the *Rhytidolepis* type are very abundant in several localities, but are too badly preserved to permit of specific determination.

Stigmariu, BRONGNIART, 1822. ('Sur. Class. végét. foss.,' p. 9.)

Stigmaria ficoides (STERNBERG).

1886. *Stigmaria ficoides*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 611, Plate 91, figs. 1-6.

1887. *Stigmaria ficoides*, WILLIAMSON, 'Palæont. Soc.,' vol. 40, pp. 1-4, 1-62, Plates 1-15.

Several specimens of *Stigmaria* have been obtained from the shales at Broadstone quarry and Warmington Farm, near Bideford, and from the sandstones at Pit quarry, Abbotsham. There is also a specimen in the British Museum (V. 3561) from Northam.

CORDAITALES.

Cordaites, UNGER, 1850. ('Gen. et Spec. Plant. Foss.,' p. 277.)

Cordaites (Artisia) sp.

1837. *Sternbergia approximata*, LINDLEY and HUTTON, 'Foss. Flora,' vol. 3, Plates 224 and 225.

1886. *Artisia approximata*, ZEILLER, 'Flore foss. bass. houill. Valenciennes,' p. 634, Plate 94, figs. 2 and 3.

The only Cordaitan remains which have been obtained from the Culm Measures were badly preserved pith casts, known as *Artisia* or *Sternbergia*, collected

from the sandstones at Broadstone quarry, Bideford, and from a small quarry above Westward Ho !.

Incertæ sedis.

Trigonocarpus, BRONGNIART, 1828. ('Prodr. Hist. végét. foss.,' p. 137.)

Trigonocarpus parkinsoni, BRONGNIART.

1835. *Trigonocarpum Næggerathi*, LINDLEY and HUTTON, 'Foss. Flora,' vol. 2, Plate 142c.

1836. *Trigonocarpum oliveforme*, LINDLEY and HUTTON, *ibid.*, vol. 3, Plate 222, figs. 1 and 3.

The only specimen of this seed known from the Culm Measures was obtained from the sandstones of Pit quarry, Abbotsham, and is in Mr. ROGERS's collection.

Bark-like Impression of a Fossil Plant. Plate 20, fig. 12.

The magnified photograph reproduced on Plate 20, fig. 12, is taken from one of several specimens of the bark-like impression of a fossil plant, obtained by Mr. ROGERS from the carbonaceous shales at Hiscott, and at Broadstone quarry, Bideford. The bark is ornamented by fairly regular "cross hatching," or striae, more or less at right angles to one another. The occurrence of leaf or of branch scars has never been observed on any specimens. The scar-like prominences seen in the photograph are entirely due to imperfections in the preservation. Mr. KIDSTON has obtained similar specimens from other British coal-fields. At present, however, we are quite without evidence as to the genus or class of plant to which these bark-like structures belong. They may possibly be impressions of the sub-epidermal tissues of partially decorticated stems, which would account for the absence of leaf scars.

B. THE PALÆOBOTANICAL EVIDENCE WITH REGARD TO THE AGE OF THE CULM MEASURES IN NORTH-WEST DEVON.

V. *Previous Opinion.*

The first definite evidence as to the age of the "anthracite or culm" beds in North-west Devon was brought forward by DE LA BECHE in 1834. As has been already stated, the plants collected by DE LA BECHE from the coal-bearing beds near Bideford were determined by LINDLEY, and in a letter communicated to the Geological Society at the end of that year, it was stated that the specimens were "plants of the coal measures." *

In 1836, SEDGWICK and MURCHISON,† in a preliminary note on the classification of

* DE LA BECHE (34), p. 106.

† SEDGWICK and MURCHISON (36), pp. 95 and 96.

the rocks of North Devon, showed that the Culm Measures, "which had been previously regarded as belonging to one of the lowest portions of the grauwacke formation," may "without hesitation be referred to the regular carboniferous series." The abstract of their paper* before the Geological Society, published in 1837, contains a list of Carboniferous plants from the Bideford district as has already been noticed.

In the magnificent treatise on the "Physical Structure of Devonshire" by the same authors, which was read before the Geological Society in June, 1837, and published three years later in the 'Transactions,' the Carboniferous age of the Culm Measures was fully demonstrated. Although expressing some uncertainty as to the particular horizon in the Carboniferous to which their lower division of the Culm Measures belonged, they had no hesitation in placing the upper series, in which the bands of "anthracite or culm" occur, in the Upper Carboniferous. "But, we repeat, no such ambiguity attaches to the upper part of the culm series; as all the vegetable fossils derived from it, are identical with published species, before obtained, from the true coal measures overlying the mill-stone grit." "The upper culm strata of Devon are the geological equivalents of the ordinary British coalfields."†

The correctness of the identification of the plants from the Upper Culm Measures was further emphasised by MURCHISON in his 'Silurian System,' published in 1839. He‡ states that "several of the plants common to the Salopian and Pembroke coalfields are the *most abundant* species in the *culm measures of Devon*." On this point, Professor LINDLEY, after a re-examination of many specimens, expressed the following opinion: "Respecting the Devon culm plants, I have still the same observation to make as before. I have looked them over carefully, and *I do not see one single species* which might not have been met with at Newcastle, with the exception of two round compressed bean-like bodies, which, if of vegetable origin, are unknown to me."§

DE LA BECHE|| in his 'Report on the Geology of Cornwall and Devon,' also published in 1839, discussed the evidence as to the age of the Upper Culm Measures, and gave reasons for his belief that fossil plants afford little or no clue to the stratigraphical horizon of the beds in which they occur. His position in this matter was, however, explained by LYELL¶ in his Presidential address to the Geological Society in 1837. SEDGWICK and MURCHISON's conclusions were fully accepted by LYELL, and also by AUSTEN,** WEAVER†† and others, although there were still a few,

* *Ibid.* (37), p. 562.

† SEDGWICK and MURCHISON (40), pp. 684 and 682.

‡ MURCHISON (39), p. 380, footnote; see also p. 85 and p. 374, footnote.

§ MURCHISON (39), p. 380, footnote.

|| DE LA BECHE (39), pp. 131-139.

¶ LYELL (37), pp. 490-496.

** AUSTEN (42), pp. 458-461.

†† WEAVER (38), p. 589.

especially WILLIAMS,* who strongly upheld the earlier view of the pre-Carboniferous age of these deposits.

For more than a quarter of a century after the publication of SEDGWICK and MURCHISON's treatise, no attention seems to have been devoted to the Upper Culm Measures in North Devon. In 1866, however, a local geologist, the late Mr. TOWNSEND HALL,† who possessed an intimate acquaintance with the Culm Measures of the Bideford district, stated that he regarded these beds as the equivalents of the Millstone Grit in other areas. In subsequent papers‡ he maintained this view, namely, that their age was immediately antecedent to that of the true Coal Measures.

PENGELLY, in his Presidential address to the Devonshire Association in 1867, stated that the Carboniferous beds of Devonshire "are admitted on all hands to be the equivalents of the Coal-measures,"§ A similar conclusion was expressed by HOLL|| with regard to the South Devon Culm Measures.

In 1872, the fifth edition of MURCHISON's 'Siluria' was published, and the various petrological types of the Culm Measures are here referred to definite horizons in the Carboniferous. The limestones of Swimbridge and Venn represent a part of the Mountain or Carboniferous Limestone. The Coddon Hill beds¶ stand in place of the Millstone Grit, and the overlying Culm are the equivalents of some of the lower coal-bearing strata in other areas.**

In the fourth edition of 'The Coal-fields of Great Britain,' Professor HULL speaks of "the flora of the 'Culm' belonging to the Lower Carboniferous series of Devonshire."††

Mr. H. B. WOODWARD‡‡ in the second edition of 'The Geology of England and Wales' published in 1887, speaking of the Devonshire Culm Measures says "some authorities have placed them, generally, on the horizon of the Millstone Grit, but there seems to be good reason to include with them representatives of at least a portion of the true Coal-measures, and also of the Carboniferous Limestone and Lower Limestone Shales. The following general divisions may be made in the Series :—

* WILLIAMS (35), (37), (39), (40), (42).

† HALL (66).

‡ HALL (75), p. 368, and (78).

§ PENGELLY (67), p. 15.

|| HOLL (68), p. 414.

¶ These beds were shown to be of Lower Carboniferous age by Messrs. HINDE and FOX in 1895.

** MURCHISON (72), pp. 272 and 273.

†† HULL (81), p. 64.

‡‡ WOODWARD (87), p. 187.

	Thick even-bedded grey grits with slates and shales. Sand- stones, grits and shales, with beds of culm.
{ Lower Coal-measures? ... }	
Culm-measure Series -; Millstone Grit ?	Coddon Hill Beds (local).
{ Lower Carboniferous? ... }	Shales, with thin grits, imper- sistent limestones and occasional bands of culm."

In more recent times, however, there would seem to be a tendency in certain quarters to regard the Culm Measures as essentially a Lower Carboniferous series of deposits. The opinion of Professor HULL, already quoted, is a case in point. This view is not, however, shared by all British Geologists. It has arisen, partly from the fact that the older literature has to some extent been lost sight of, and partly from the interest aroused by the recent work of Messrs. HINDE and FOX, which placed our knowledge of the Lower Carboniferous portion of the Culm Measures on an altogether different footing to any which we possess of the Upper Culm Measures. We have also in recent years gained a more intimate acquaintance with the flora of the so-called culm, or kuhn of Germany, Austria, and elsewhere on the Continent; deposits of Lower Carboniferous age as shown by the character of their fossil remains. The identification by Mr. KIDSTON* of certain Lower Carboniferous species of plants believed to have been obtained from the Culm Measures, but which, as already shown, were mostly derived from the Upper Devonian, has been quoted by WOODWARD,† USSHER‡ and others in this connection.

It is one of the main objects of the present paper to show that the view that the Culm Measures as a whole are essentially of Lower Carboniferous age is incorrect.

Lastly, the opinion of Mr. USSHER, who has by far the widest acquaintance with the Culm Measures of any living geologist, and who has devoted many years to their exploration on behalf of the Geological Survey, may be quoted as having considerable weight. In his earlier papers,§ Mr. USSHER does not offer any opinion as to the horizons in the Upper Carboniferous represented in Devon, and in 1888, he|| remarks "that he doubts the value of any correlation of these divisions with those of the Carboniferous of other parts of England, but gives the following for what it is worth."

* KIDSTON (84¹) and (84²).

† WOODWARD (87), p. 197.

‡ USSHER (92), p. 160.

§ USSHER (79), (86), (87).

|| USSHER (88), p. 141.

Upper Culm Measures	= ? Lower Coal measures or Millstone Grit.
Middle Culm Measures	} Millstone Grit.
Lower Culm Measures { St. David's type Coddon Hill beds and impersistent limestones.	
	} Carboniferous Limestone.

In his more recent papers,* published in 1892 and 1901, Mr. USSHER apparently inclines to the opinion that the higher divisions of the Culm Measures are the equivalents of the Millstone Grit, although he does not express any very decided views.

VI.—*The Middle Coal Measures of the Bideford District.*

We have seen from the summary of previous opinion with regard to the Upper Culm Measures, that the age of these beds was first determined as Upper Carboniferous, by DE LA BECHE, and by SEDGWICK and MURCHISON, entirely on the evidence of the fossil flora; a striking illustration of the contributions of Palæobotany to Stratigraphical Geology.

In recent years, we have come to know more of the distribution of fossil plants in the Coal Measures of this country, chiefly as the result of Mr. KIDSTON's researches,† and it is now possible to distinguish clearly a certain number of horizons in the Upper Carboniferous, by the character of the flora. The tests applied are, as I have recently explained elsewhere,‡ entirely different from those made use of in zoning the Lower Palæozoic and Jurassic rocks by means of the invertebrate remains. At the beginning of Upper Carboniferous times, a marked change in the character, but not in the general relationships of the flora, took place. So marked was this break that, although, so far as we are aware, most of the genera of both Upper and Lower Carboniferous rocks belong to the same families and classes, yet no species of reliable value is known to occur in both these great divisions.

During Upper Carboniferous and Permian times, periods which belong to the same botanical epoch, and are somewhat sharply marked off botanically from the Lower Carboniferous and Upper Devonian, the general character of the flora in its broad outlines remained constant. Yet detailed study has shown that it is possible to detect the changes which took place during this interval, involving the gradual coming in or dying out of genera and species, and the maximum development of now one genus and now another. That a considerable change did take place is evident from a comparison of the two extremes of these series, the Permian flora of the Continent, now known in great detail, and the flora of the British Lower Coal Measures.

* USSHER (92), p. 179, and (01), p. 386.

† KIDSTON (93).

‡ ARBER (03).

The whole principle of zoning the Carboniferous system by means of fossil plants, whether in this country or abroad, depends upon the recognition of definite periods in this change, periods in which the change itself has become rather marked. Many species extend throughout the Upper Carboniferous, but this is by no means the case with all. Some genera, and many species have a far shorter vertical distribution than others, and their presence gives a dominant character to the flora at any one horizon. By themselves, these species are unreliable as evidence, if only from the danger of negative evidence, but by taking into account the aggregate flora at any one horizon the evidence gained is found to be trustworthy.

Briefly, we can so far distinguish four palæobotanical sub-divisions of the Upper Carboniferous.

Examples.

- | | | |
|-----------------------------|---|---|
| (4) Upper Coal Measures . | { | Radstock and Farrington Series, Somerset.* |
| | | Upper Pennant Series, South Wales.† |
| (3) Upper Transition Series | { | Lower Pennant Series, South Wales.† |
| | | Ardwick Series, Manchester. |
| (2) Middle Coal Measures . | . | Dudley Coalfield, South Staffordshire. |
| (1) Lower Coal Measures . | . | Gannister beds of Lancashire and Yorkshire. |

Returning to the Upper Culm Measures of the Bideford district, the following species have been found to occur, and their distribution in the Upper Carboniferous of Britain is as follows:—

Distribution of Upper Carboniferous Species which occur in the Upper Culm
Measures of the Bideford District.

× = recorded, × × = especially abundant on this horizon, (×) = rare on this horizon.

Species.	Lower Coal Measures.	Middle Coal Measures.	Upper Transition Series.	Upper Coal Measures.
Equisetales—				
<i>Calamites (Calamitina) undulatus</i> , STERNB. .	× ×	× ×		(×)
<i>C. (Eucalamites) ramosus</i> , ARTIS	× ×	× ×	×	×
<i>C. (Stylocalamites) suckowii</i> , BRONG. . . .	× ×	× ×		×
<i>C. (Calamitina) varians</i> , STERNB.	×	×		
<i>Annularia radiata</i> , BRONG.	× ×	× ×		
<i>A. galloides</i> (L. and H.)	×	×		
<i>Calamocladus equisetiformis</i> (SCHL.) . . .	× ×	× ×	×	× ×
<i>C. charaformis</i> (STERNB.)		×	×	
<i>Calamostachys longifolia</i> , WEISS.	×	×		
Sphenophyllales—				
<i>Sphenophyllum cuneifolium</i> (STERNB.) . .	× ×	× ×	×	

* Kidston (88).

† Kidston (94).

Distribution of Species—*continued*.

Species.	Lower Coal Measures.	Middle Coal Measures.	Upper Transition Series.	Upper Coal Measures.
<i>Cycadofilices</i> —				
<i>Neuropteris obliqua</i> (BRONG.)	x	x		
<i>N. schlechani</i> , STUR. . . .		x		
<i>Althopteris sortii</i> (BRONG.) .		(x)	(x)	x x
<i>A. lonchitica</i> (SCHL.) . . .	x x	x x	(x)	(x)
<i>Filicales</i> ?—				
<i>Mariopteris muricata</i> (SCHL.)	x x	x x		(x)
<i>Urnapteris tenella</i> (BRONG.)	x	x		
<i>Renaultia footneri</i> (MARRAT)	x	x		
<i>Lycopodiales</i> —				
<i>Lepidolendron aculeatum</i> , STERNB.	x x	x x		x x
<i>L. obovatum</i> , STERNB.	x x	x x		
<i>L. fusiforme</i> (CORDA).	x	x		x
<i>Lepidophloios acerossus</i> (L. and H.)	x	x	x	
<i>Stigillaria scutellata</i> , BRONG. . .	x	x	x	
<i>S. tessellata</i> , BRONG.	(x)	x x	x	
<i>Stigmarna foveolatus</i> (STERNB.). . .	x x	x x	x x	x x
<i>Incerte Sedis</i> —				
<i>Trigonocarpus parkinsoni</i> , BRONG. .	x	x	x	

*Additional Generic, and doubtful Specific Determinations.**Equisetales*.—*Calamites* sp. (external surface).*Pinnularia* sp.*Filicales* ?—*Megalopteris* sp ?*Renaultia schatzlauerensis* (STUR.) ?*Cordaitales*.—*Cordaites* (*Artisia*) sp.*Incerte Sedis*.—

Bark-like impression.

The general character of this flora is identical with that developed elsewhere in Britain in the Middle Coal Measures. As will be seen from the above table, the majority of the species determined are known to occur in both the Lower and Middle Coal Measures. But there are also present several which have, so far, never been found on the lower horizon. The flora of the Middle Coal Measures is everywhere distinguished by the occurrence of such species. In other words, the change in the

general character of the flora of the Upper Carboniferous, as traced from the base to the summit, is already marked on this horizon by the presence of new species which are not found in the lowest beds. *Neuropteris schlehani*, *Alethopteris serli*, and *Calamocladus chareiformis* are unknown from the Lower Coal Measures, while *Sigillaria tessellata* and *Neuropteris obliqua* are markedly more abundant in the Middle than the Lower Coal Measures. The common occurrence of *Sigillaria*, a genus which reaches its maximum in the Middle Coal Measures, and which is neither frequent nor represented by many species on the lower horizon, adds confirmatory support to this conclusion.

This flora is further distinguished from that of the Upper Transition Series and the Upper Coal Measures by the entire absence of certain genera or species, especially *Pecopteris* and its allies, which are characteristic of those horizons, as well as by the fact that many of the species described here are either entirely unknown from, or of very rare occurrence in the higher beds.

The main conclusion of this paper is, therefore, that the coal-bearing horizon in the Upper Culm Measures, developed in the Bideford district, is equivalent to the Middle Coal Measures in other British coalfields.

VII. General Conclusions.

It has been already pointed out that the thickness and extent of the Upper Culm Measures of SEDGWICK and MURCHISON (a division which practically includes the Middle and Upper Culm Measures of Mr. USSHER's* classification), is far greater than that of the Lower Culm Measures.†

Thus there can be no doubt that the Culm Measures of Devon, as a whole, are essentially of Upper Carboniferous age, although Lower Carboniferous beds are also represented. The age of the latter series was determined by Messrs. HINDE and FOX in 1895, as the result of an examination of a large and varied fauna, more especially derived from the Coddon Hill series. As might be expected from the marine character of these beds, few fossil plants have ever been obtained from them.‡

Above the chert deposits lie the great series of the shales and sandstones which constitute the Upper Culm Measures. If we follow the valley of the Torridge from its junction with the Taw, as far south as Torrington, we pass directly across the line of strike of the Upper Culm Measures, from the lowest to the highest, or approximately the highest beds.

At Instow Quay, a half-mile to the south of the junction of the two rivers, we find

* USSHER (01), p. 362.

† See SEDGWICK and MURCHISON (40), p. 677; HINDE and FOX (95), p. 662; and Mr. USSHER's map (01), Plate 16.

‡ A few doubtful species have been recorded from the limestones (see USSHER (92), pp. 124, 127, 128, and 130).

the first link in the chain of evidence. The beds here consist of shales alternating with sandstones, the former containing many distinctly calcareous nodules. It was from these shales and nodules that the late TOWNSHEND HALL,* in 1876, recorded the fish and Goniatite remains, which are now preserved in the British Museum (Natural History). The fish remains are esturine, and belong to the following species :—

Calacanthus elegans, NEWB.†
Elonichthys aitkeni, TRAQ.‡

Both of these are known only from the Upper Carboniferous, and occur in the Lower Coal Measures, although they are not confined to that horizon.§

In addition, the following Cephalopoda have been found in the calcareous shales :—

Gastrioceras carbonarium (VON BUCH).||
Gastrioceras Listeri (MARTIN).¶

Both these species are, I believe, abundant in the Lower Coal Measures in this country. It may be mentioned that since HALL's specimens are much water-worn, and might conceivably give rise to the suspicion that these shales were not found in place, I have had the Instow beds carefully re-examined, with the result that further specimens of the Goniatites, and also specimens of *Pterinopecten* (*Aviculopecten*) *papyraceus*, (Sow.) have been obtained, which were undoubtedly *in situ*. The only plant remains which have been found at Instow are imperfect Calamitean casts.

These discoveries afford strong evidence that the Culm Measures at Instow belong to the lowest portion of the Upper Carboniferous ;** an horizon known to the palæobotanist as the Lower Coal Measures.††

Passing some 2½ miles to the south of Instow, we reach, at Bideford, a higher horizon in the Culm Measures, which has been shown here to be the equivalent of the Middle Coal Measures in other British areas. These beds are of a

* HALL (76), p. 410; see also HINDE and FOX (95), p. 655.

† Registered numbers in Geological Department, P. 5379 and P. 6268.

‡ P. 6100.

§ TRAQUAIR (03), p. 700.

|| C. 1613.

¶ C. 1613A.

** Messrs. HINDE and FOX (95), p. 663, however, have expressed a doubt as to whether these beds should be included in the Upper Culm Measures.

†† The Millstone Grit is no longer regarded as a separate horizon, either by the palæobotanists, or the palæozoologists who are working at the distribution of carboniferous life, for both its flora and fauna are indistinguishable from that of the Lower Coal Measures.

shallow water or estuarine facies, as is shown by the abundance of plant remains. In addition to the plants described here, the following mollusca, kindly determined for me by Dr. WHEELTON HIND, were obtained at Bideford in association with plant remains :—

Carbonicola acuta (Sow.).

C. aquilina (Sow.)?

So far we have seen that there are good reasons for the belief that both the Lower and Middle Coal Measures are represented in Devon. With regard to the higher beds developed near Torrington, and along the northern and western coast lines in the neighbourhood of Clovelly and Hartland, there is no evidence at present as to the position which they occupy in the Upper Carboniferous series. So far, I have hardly obtained any plants from these beds, but it is hoped that a more thorough and extended examination, which is shortly to be undertaken, will yield evidence as to the horizon to which they belong. This portion of the Culm Measures has never been studied in detail, and remains practically virgin ground for the geologist.* The highest beds are termed by Mr. USSHER† the Eggesford Grits or Upper Culm Measures, in his three-fold classification. I have had some opportunity of examining these rocks, but I have found it almost impossible to distinguish them clearly by their lithological or physical characters from the shales and sandstones of the Morchard type (Middle Culm Measures of Mr. USSHER), from which the plants described here were obtained. For this reason the original two-fold division of the Culm Measures into Upper and Lower, instituted by SEDGWICK and MURCHISON, has been maintained here.

It would be out of place in a paper, which is botanical rather than geological, to attempt to institute a detailed comparison between the lithological characters of the Carboniferous sequence in Devon, and those of other Carboniferous areas in Britain or on the Continent. There are, however, two points which may be briefly discussed. While the Lower Carboniferous portion of the Culm Measures, with its comparatively feeble development of calcareous deposits, and its considerable thickness of Radiolarian cherts, differs as a whole somewhat markedly from the Lower Carboniferous rocks of many other British areas,‡ this is not the case in the same degree with the Upper Carboniferous division. The sandstones and shales of the Upper Culm Measures recall somewhat vividly those of certain other English coalfields. The presence of numerous plant remains in this division of the Culm Measures points to conditions of

* So far as I am aware, the only literature, with the exception of Mr. USSHER's papers, is a short note by CONYBEARE (14), published in 1814, on the Culm Measures near Clovelly.

† USSHER (01), pp. 362 and 366.

‡ It has been long known that rocks of similar petrological character to the Lower Culm Measures of Devon occur in the upper portion of the Lower Carboniferous sequence ("Gower Series" or "Bishopston Beds") in South Wales. See DE LA BECHE (46), p. 133, &c., and WOODWARD (87), pp. 167 and 172.

deposition not wholly dissimilar to those which pertained in other areas. The occasional occurrence of marine calcareous bands,* which has now been demonstrated on more than one horizon in Devonshire, is a feature common to other British coalfields, more especially those in North Staffordshire, and South Lancashire. The general absence of carbonaceous deposits in Devon remains the most remarkable feature of these beds, and in this respect the Culm Measures of Devon are sharply contrasted with other Carboniferous areas in England.

It is peculiarly unfortunate, as Professor LEBOUR† pointed out some years ago, that the term Culm or Kulm has become generally applied to certain series of deposits more especially developed in Germany and Austria.

SEDGWICK and MURCHISON were the first, as we have seen, to adopt the Devonshire name for the impure coal occurring near Bideford, and to institute the term Culm Measures. These authors,‡ at the conclusion of their researches in Devon, examined the Carboniferous rocks of Westphalia and other areas in Germany, and instituted a close comparison with the Culm Measures. Since then, the term Culm seems to have been adopted by most Continental geologists. Yet these deposits, however much they may physically resemble the Devonshire rocks, are of different ages. The comparison instituted by SEDGWICK and MURCHISON between the British and German rocks, and supported in more recent times by Mr. USSHER,§ and MESSRS. HIND and FOX,|| is no doubt true of that portion of the Devonshire Culm Measures which is of Lower Carboniferous age, but this, as has been already pointed out, is now known to represent a comparatively small fraction of the Carboniferous series of Devon and the adjacent counties; a series which, as a whole, is essentially Upper, and not Lower Carboniferous in age. On the other hand, the sequence of so-called Culm or Kulm deposits in Silesia, Moravia, the Hartz, Westphalia and elsewhere in Germany, has yielded a series of plant remains which are now well known from the researches of STUR, GÖPPERT, POTONÍ and others, and which are typically and entirely of a Lower Carboniferous facies.

Professor LEBOUR¶ has suggested that the Germanised form of the word Culm, *i.e.*, Kulm, should be adopted for the Continental deposits exclusively, in order to minimise the confusion which must constantly arise with regard to these two non-contemporaneous series. It would seem to me better, if possible, to drop the term Culm Measures altogether in speaking of the Devon Carboniferous rocks, and to apply the nomenclature adopted with regard to other British areas of similar age, which has been shown here to be applicable to Devonshire, at least with regard to the

* HALL (76), and ROGERS and ARBER (04).

† LEBOUR (79), p. 352.

‡ SEDGWICK and MURCHISON (42), p. 276.

§ USSHER (92), p. 169; (01), p. 374.

|| HIND and FOX (95), p. 658.

¶ LEBOUR (79), p. 353.

Upper Carboniferous deposits. As, however, this suggestion may not be altogether practicable, I have retained the term "Culm Measures" in this paper in order to avoid confusion.

BIBLIOGRAPHY.

- ANDREWS, E. B. (75). "Descriptions of Fossil Plants from the Coal Measures of Ohio," 'Rep. Geol. Surv., Ohio.' *Palæontology*, pt. 2, p. 413, 1875.
- ARBER, E. A. N. (03). "The use of Carboniferous Plants as Zonal Indices," 'Trans. Inst. Min. Engineers,' vol. 25, p. 371, 1903.
- (04). See ROGERS and ARBER.
- AUSTEN, R. A. C. (42). "On the Geology of the South-east of Devonshire," 'Trans. Geol. Soc.,' Ser. 2, vol. 6, Part 2, p. 433, 1842.
- CONYBEARE, J. J. (14). "Memoranda relative to Clovelly, North Devon," 'Trans. Geol. Soc.,' Ser. 1, vol. 2, p. 495, 1814.
- DAWSON, J. W. (73). "Report on the Fossil Plants of the Lower Carboniferous and Millstone Grit," 'Geol. Surv. Canada,' 1873.
- DE LA BECHE, H. T. (34). "On the Anthracite found near Bideford in North Devon," 'Proc. Geol. Soc.,' vol. 2 (No. 37), p. 106, 1834-35.
- (39). 'Report on the Geology of Cornwall, Devon, and West Somerset.' London, 1839.
- (46). "On the Formation of the Rocks of South Wales and South Western England," 'Mem. Geol. Survey, Great Britain,' vol. 1, p. 1, 1846.
- FOX, H. See HINDE and FOX.
- HALL, T. M. (66). "The Geology of North Devon" ('A Lecture to the Exeter Naturalists Club,' September 23, 1865), Priv. Print., 1866.
- (75). "Notes on the Anthracite Beds of North Devon," 'Rep. and Trans. Devon Ass.,' vol. 7, p. 367, 1875.
- (76). "Fossil Fish in North Devon," 'Geol. Mag.,' Dec. 2, vol. 3, p. 410, 1876.
- (78). "A Sketch of the Geology of Devonshire," in WHITE'S 'History, Gazetteer and Directory of the County,' p. 58, 1878-9. Third Edition, 1890.
- HINDE, G. J. and FOX, H. (95). "On a Well-marked Horizon of Radiolarian Rocks in the Lower Culm Measures of Devon, Cornwall, and West Somerset," 'Quart. Jour. Geol. Soc.,' vol. 51, p. 609, 1895.
- HOLL, H. B. (68). "On the Older Rocks of South Devon and East Cornwall," 'Quart. Journ. Geol. Soc.,' vol. 24, p. 400, 1868.
- HULL, E. (81). 'The Coal-fields of Great Britain.' London, Fourth Edition, 1881.

- KIDSTON, R. (84¹) in WOODWARD, H. "On the Discovery of Trilobites in the Culm-Shales of South-East Devonshire," 'Geol. Mag.,' N.S., Dec. 3, vol. 1, footnote on p. 534, 1884.
- (84²) in WOODWARD, H. "A Monograph of the British Carboniferous Trilobites," 'Paleont. Soc.,' footnote on p. 59, 1884.
- (88). "On the Fossil Flora of the Radstock Series of the Somerset and Bristol Coal Field," 'Trans. Roy. Soc.,' Edinb., vol. 33, part 2, p. 335, 1888.
- (90). "The Carboniferous Flora of Yorkshire," 'Reports 1-6, Geol. Trans. Yorks. Nat. Union,' 1890-98.
- (91). "Notes on the Palæozoic Species mentioned in LINDLEY and HUTTON's "Fossil Flora," 'Proc. Roy. Phys. Soc.,' Edinb., vol. 10, p. 345, 1891.
- (93). "On the Various Divisions of British Carboniferous Rocks as determined by their Fossil Flora," 'Proc. Roy. Phys. Soc.,' Edinb., vol. 12, p. 223, 1893.
- (94). "On the Fossil Flora of the South Wales Coal Field, &c.," 'Trans. Roy. Soc.,' Edinb., vol. 37, Part 3, p. 565, 1894.
- LEBOUR, G. A. (79). "On 'Culm' and 'Kulm,'" 'Report Brit. Ass.,' Sheffield, pp. 352-3, 1879.
- (88). in 'Reports, Sub-Committees on Classification and Nomenclature, International Geological Congress,' Cambridge. Second Edition, p. 154, 1888.
- LYELL, C. (37). "Anniversary Address to the Geological Society, 1837," 'Proc. Geol. Soc.,' vol. 2 (No. 49), p. 479, 1837.
- McMAHON, C. A. (90). "Notes on the Culm-Measures at Bude, North Cornwall," 'Geol. Mag.,' Dec. 3, vol. 7, p. 106, 1890.
- MURCHISON, R. I. (39). 'The Silurian System.' London, 1839.
- (72). 'Siluria.' London. Fifth Edition, 1872.
- See also SEDGWICK and MURCHISON.
- PENGELLY, W. (67). "The present position of Opinion respecting the Geology of Devonshire," 'Rep. and Trans. Devon Ass.,' vol. 2, p. 1, 1867.
- PHILLIPS, J. (41). 'Figures and Descriptions of the Palæozoic Fossils of Cornwall, Devon, and West Somerset.' London, 1841.
- ROGERS, I. and ARBER, E. A. N. (04). "Note on a new Fossiliferous Limestone in the Upper Culm Measures of West Devon," 'Geol. Mag.,' Dec. 5, vol. 1, p. 305, 1904.
- SEDGWICK, A. and MURCHISON, R. I. (36). "A Classification of the old Slate Rocks of the North of Devonshire," 'Report Brit. Ass.,' Bristol (ii), p. 95, 1836.
- (37). "On the Physical Structure of Devonshire, and on the sub-divisions and geological relations of its

- old stratified deposits," 'Proc. Geol. Soc.,' London, vol. 2 (No. 51), p. 556, 1837.
- SEDGWICK, A., and MURCHISON, R. I. (40). "On the Physical Structure of Devonshire, and on the subdivisions and Geological Relations of its older stratified Deposits, &c.," 'Trans. Geol. Soc.,' Series 2, vol 5, Part 3, p. 633, 1840.
-
- (42). "On the Distribution and Classification of the older or Palæozoic Deposits of the North of Germany and Belgium, and their comparison with Formations of the same age in the British Isles," 'Trans. Geol. Soc.,' Ser. 2, vol. 6, Part 2, p. 221, 1842.
- TRAQUAIR, R. H. (03). "The Distribution of Fossil Fish-remains in the Carboniferous Rocks of the Edinburgh District," 'Trans. Roy. Soc.,' Edinb., vol. 40, Part 3, p. 687, 1903.
- USSHER, W. A. E. (79). "On the Geology of Parts of Devon and West Somerset," 'Proc. Somersetshire Arch. and Nat. Hist. Soc.,' N.S., vol. 5, 1879.
-
- (86). "The Culm Measures of Devonshire," 'Brit. Ass. Report,' Birmingham, p. 676, 1886.
- (87). "The Culm Measures of Devonshire," 'Geol. Mag.,' New. Ser., Dec. 3, vol. 4, p. 10, 1887.
- (88). in 'Reports, Sub-committees on Classification and Nomenclature, International Geological Congress,' Cambridge, Second Edition, 1888.
- (92). "The British Culm Measures," 'Proc. Somersetshire Arch. and Nat. Hist. Soc.,' vol. 38, p. 111, 1892.
-
- (01). "The Culm-Measure Types of Great Britain," 'Trans. Inst. Mining Engineers,' vol. 20, p. 360, 1901.
-
- (02). "The Geology of the Country around Exeter," 'Mem. Geol. Surv.,' 1902.
- WEAVER, T. (38). "Geological Relations of North Devon," 'Proc. Geol. Soc.,' vol. 2 (No. 53), p. 589, 1838.
-
- (39). "On the Older Stratified Rocks of North Devon," 'Lond. and Edinb. Phil. Mag.,' Ser. 3, vol. 15, p. 109, 1839.
- WILLIAMS, D. (35). "On certain Fossil Plants from the opposite Shores of the Bristol Channel," 'Rep. Brit. Ass.,' vol. 5. Dublin (Pub. 1836), (ii) p. 63, 1835.
-
- (37). "On some Fossil Wood and Plants recently discovered by the Author of this Memoir, low down in the Grauwacke of Devon, &c.," 'Rep. Brit. Ass.,' vol. 6. Liverpool (Pub. 1838), (ii) p. 94, 1837.
-
- (39). "On as much of the Transition or Grauwacke system as is exposed in the counties of Somerset, Devon and Cornwall," 'Proc. Geol. Soc.,' vol. 3 (No. 63), p. 115, 1839.

- WILLIAMS, D. (40). "On as much of the great Grauwacke System as is comprised in the group of West Somerset, Devon and Cornwall," 'Proc. Geol. Soc.,' vol. 2 (No. 66), p. 158, 1840.
- (42). "Plausible Reasons, &c., showing that no Portion of the Devonian System can be of the age of the Old Red Sandstones," 'Lond. and Edinb. Phil. Mag.,' Ser. 3, vol. 20, p. 117, 1842.
- WOODWARD, H. B. (87). 'The Geology of England and Wales,' London, Second Edition, 1887.

EXPLANATION OF THE PLATES.

(Photographs by Mr. TAMS, Cambridge.)

PLATE 19.

Fig. 1. *Calamites (Calamitina) undulatus*, STERNB.

A portion of a pith cast showing a node, and two internodes. The sinuous character of the longitudinal ridges is well seen, also the infranodal canals, and the wedge-like endings of the ridges at the node. Very slightly enlarged. (Mr. ROGERS's Coll. No. 18, from the cliff above Bideford railway station.)

Fig. 2. *Calamites (Eucalamites) ramosus*, ARTIS.

A portion of a pith cast showing part of an internode, and a large branch scar at the node. Natural size. (Mr. ROGERS's Coll. No. 12, from the cliff above Bideford railway station.)

Fig. 3. *Calamites (Calamitina) undulatus*, STERNB.

A pith cast showing two nodes. The longitudinal ridges on the internodes are sinuous. The terminations of the ridges and the infranodal canals below the nodes are clearly seen. Reduced to half natural size. (Mr. ROGERS's Coll. No. 11, from the cliff above Bideford railway station.)

Fig. 4. *Sigillaria scutellata*, BRONG.

Natural size. (Mr. ROGERS's Coll. No. 10, from the sandstones of Pit (ROGERMAN's) quarry, Abbotsham, near Bideford.)

Fig. 5. *Lepidodendron fusiforme* (CORDA).

$\times 2\frac{1}{2}$. (Mr. ROGERS's Coll. No. 54, from Broadstone quarry, East-the-Water Bideford.)

Fig. 6. *Urnatopteris tenella* (BRONG.).

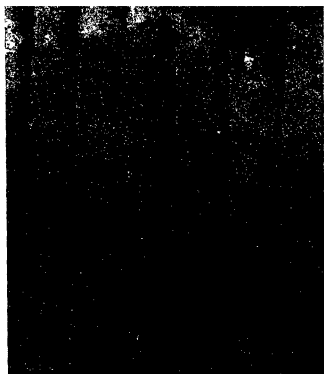
Photograph from a drawing, $\times 2\frac{1}{2}$. (Mr. ROGERS's Coll. No. 20, from ROBERTS's quarry, East-the-Water, Bideford.)

Fig. 7. *Sigillaria tessellata*, BRONG.

Natural size. (Mr. ROGERS's Coll. No. 39, from the Culm bands at Hiscott, near Tawstock.)



1



4



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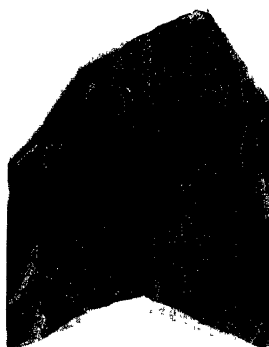
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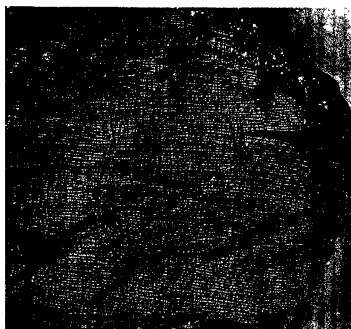


Fig. 8. *Lepidodendron aculeatum*, STERNB.

Natural size. (Mr. ROGERS's Coll. No. 21, from the Culm bands at Hiscott, near Tawstock.)

Fig. 9. *Lepidophloios acerosus* (L. and H.).

Slightly enlarged. (Mr. ROGERS's Coll. No. 40, from the Culm bands at Hiscott, near Tawstock.)

PLATE 20.

Fig. 10. *Neuropteris schlehani*, STUR.

A fragment of a pinna, showing the form and nervation of the pinnules. $\times 3\frac{1}{4}$. (Mr. ROGERS's Coll. No. 50, from Broadstone quarry, East-the-Water, Bideford.)

Fig. 11. *Alethopteris lonchitica* (SCHL.).

Natural size. (HALL Coll. in the Athenæum, Barnstaple, from Alverdiscott.)

Fig. 12. Unknown Bark of a plant. $\times 3\frac{1}{2}$. (Mr. ROGERS's Coll., from Broadstone quarry, East-the-Water, Bideford.)

Fig. 13. *Neuropteris schlehani*, STUR.

A fragment of a pinna, showing the nervation. $\times 3\frac{1}{2}$. (Mr. ROGERS's Coll. No. 51, from Broadstone quarry, East-the-Water, Bideford.)

Fig. 14. *Alethopteris serli* (BRONG.).

$\times 3\frac{1}{8}$. (Mr. ROGERS's Coll. No. 42, from the Culm bands at Hiscott, near Tawstock.)

Fig. 15. *Alethopteris lonchitica* (SCHL.).

The terminal portion of a pinna of a form or variety of this species in which the pinnules are contracted at the base, and not decurrent. Natural size. (Museum of the Geol. Surv., Jermyn Street, No. 8423, from Moor Park, Bideford.)

Fig. 16. *Megalopteris* sp. ?

A portion of a pinnule showing the thick midrib, and dichotomous secondary nervation. Slightly enlarged. (HALL Coll., in the Athenæum, Barnstaple; from Alverdiscott.)

Fig. 17. *Mariopteris muricata* (SCHL.).

$\times 2\frac{1}{2}$. (SEDGWICK Mus. Camb., Carb. Plant Coll. No. 1148; from ROBERTS's quarry, East-the-Water, Bideford.)

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Phil. Trans., B, vol. 197, 1904, pp. 327-345.

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XI. On the Action of the Venom of *Bungarus Cœruleus* (the Common Krait).

By MAJOR R. H. ELLIOT, I.M.S.* W. C. SILLAR, M.B., B.Sc.; Lecturer on Experimental Pharmacology and Senior Assistant in the Materia Medica Department of the University of Edinburgh; and GEORGE S. CARMICHAEL, M.D., University Assistant in the Materia Medica Department of the University of Edinburgh.

Communicated by Professor SIR THOMAS R. FRASER, M.D., F.R.S.

(From the Pharmacological Laboratory of the University of Edinburgh.)

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THE following experimental research upon the pharmacological action of the venom of the common Krait (*Bungarus cœruleus*), was performed in the Pharmacological Laboratory of the University of Edinburgh, and we are greatly indebted to Sir THOMAS R. FRASER, not only for facilities in the performance of that work, but also for the encouragement he has uniformly given us.

Previous examination of the action of this venom appears to be limited to the work recorded in the papers recently published by Captain LEONARD ROGERS, I.M.S.† He states that the symptoms following poisoning by this venom are identical with those following poisoning by Cobra venom, whereas our results, as shown below, seem to indicate that while these symptoms are similar, still they differ so much in relative degree as to render it doubtful if they can in future be spoken of as identical.

* On special duty for Snake Venom research under the orders of the Secretary of State for India.

† 'Indian Medical Gazette,' vol. 39, No. 1, January, 1904, p. 30; 'Lancet,' February 6, 1904, p. 350.

For the Krait venom used in the experiments we are greatly indebted to the Madras Government, who placed Dr. P. P. PINTO on special duty for the purpose of collecting venom. We take this opportunity of expressing our thanks to this gentleman, for we are aware how difficult it is to obtain specimens of this poison.

The total amount available was 0·077 of a grm., which was the venom furnished by expression from the dissected glands of two snakes. This gives a total of 0·0385 of a grm. per snake and corresponds very closely with what one of us has found to be the ordinary yield of this species. It is obviously much less than the average yield from a large Cobra.

Experiments were performed :—

- (1) To determine the minimum lethal dose of the dried venom for frogs and small mammals—rats and rabbits being chosen.
- (2) To determine the protective power of CALMETTE'S anti-venomous serum against Krait venom compared with its power of antagonising Cobra venom.
- (3) To determine whether any intravascular clotting or hæmalysis occurred in the circulating blood, following a lethal dose.
- (4) To determine the action of the venom on the isolated vessels of the frog.
- (5) To determine the action of the venom on the isolated ventricle of the frog.
- (6) To ascertain generally the causes which influence the vital functions of mammals exposed to the action of the venom and which bring about death.

EXPERIMENTS to determine the Minimum Lethal Dose of Krait Venom for Frogs (*Rana esculenta*). Solution used was 0·0001 grm. in 1 cub. centim. of RINGER'S Fluid.

No.	Dose per kilo.	Weight of frog.	Remarks.
1	grm. 0·002	kilo. 0·047	3 hours after injection, frog prone; respiration very shallow; lower eyelids fully and permanently raised. Died 22 hours after injection.
2	0·0015	0·044	2 hours after injection, frog prone; respiration very irregular; eyelids fully raised. Died within 22 hours.
3	0·001	0·041	4 hours after injection, respiration normal; eyelids three-quarters raised. 22 hours after injection respiration ceased; eyelids fully raised. Died in 3 days.
4	0·0005	0·039	3 hours after injection, eyes open; respiration easily visible. 22 hours after injection, eyelids raised; frog prone; can turn over; reflexes present; respiration shallow. Died in 3 days.

EXPERIMENTS to determine the Minimum Lethal Dose by Subcutaneous Injection of Krait Venom for Rabbits. The solution used was 0·0001 grm. in 1 cub. centim. of RINGER'S Fluid.

No.	Dose per kilo.	Weight of rabbit.	Remarks.
1	gm. 0·00125	kilo. 1·420	Died in 4 hours. The phrenic nerves responded to an induction current with the coils distant 450 millims. The vagi responded at 140 millims., and the left sciatic at 220 millims.
2	0·001	1·580	Died in 6 hours. Phrenics responded at 220 millims. Right vagus at 20 millims.; left vagus at 100 millims. Right sciatic at 220 millims.
3	0·0009	1·630	Died in 5 hours. Phrenics failed to respond with coils separated more than 60 millims. Vagi gave no complete response to maximal stimulus.
4	0·00075	1·590	Died in 2 hours.
5	0·0007	1·680	Died in 5 hours. Phrenics responded at 420 millims.; vagi at 100 millims.
6	0·0006	1·625	Died in 6 hours.
7	0·0005	1·975	Died in 4½ hours. Right phrenic responded at 420 millims.; left phrenic at 220 millims.
8	0·0004	2·220	Died in 5½ hours. Phrenics responded at 390 millims.; vagi at 80 millims.
9	0·0002	2·460	Died within 15 hours.
10	0·00015	1·375	Died within 21 „
11	0·0001	2·120	Died within 21 „
12	0·00009	2·110	Died within 21 „
13	0·00008	1·960	Died within 21 „
14*	0·00007	1·860	Recovered; moderately ill.
15	0·00006	1·840	Recovered. Very ill for 2 days; extremities paralysed.
16	0·00004	2·120	Recovered.

* The solution of venom used in Experiment 14 was 1 day old.

EXPERIMENTS to determine the Minimum Lethal Dose by Subcutaneous Injection of Krait Venom for Rats. The solution used was 0.0001 grm. in 1 cub. centim. of RINGER's Fluid.

No.	Dose per kilo.	Weight of rat.	Remarks.
1	gm. 0.004	kilo. 0.087	Died in 3 hours.
2	0.003	0.053	Died in 1½ „
3	0.002	0.079	Died in 7-9 hours.
4	0.001	0.072	Died in 3½ hours. The phrenic nerves at death failed to respond to an induction current with the coils distant more than 220 millims. The right vagus partially responded at 40 millims. Left vagus nil.
5	0.0009	0.244	Recovered after being very ill.
6	0.0008	0.153	Recovered.
7	0.0007	0.157	Recovered.
8	0.0006	0.155	Recovered.

A point of special interest is the rise of the lower eyelids. This is due to an elastic recoil of the lids when the depressor of the lower eyelids is paralysed. The interest attached to this point lies in the fact that Cobra venom and another Colubrine venom produce the same symptom, while it is invariably absent in the course of Daboia poisoning.

We have therefore concluded that the minimum lethal dose of our specimen of dried Krait venom was, in the case of the frog, about 0.0005 grm. per kilo.; in the case of the rat, 0.001 grm. per kilo., and in the case of the rabbit as low as 0.00008 grm. per kilo. Our supply of the venom was too small to allow us to determine the lethality in other animals.

The relatively great lethal action of Krait venom for the rabbit as compared with the rat is remarkable and shows a great divergence from the relative lethality of Cobra venom for the same animals as determined by one of us, who found the M.L.D. of a specimen of Cobra venom for rats 0.0005 grm. per kilo., and for rabbits 0.0006 grm. per kilo.

EXPERIMENTS to demonstrate the Protective Power of CALMETTE's Antivenomous Serum against (1) Cobra Venom and (2) Krait Venom.

The solution of dried Cobra venom in RINGER's fluid used was of a strength of 0.0001 grm. in 10 cub. centims.; and the solution of Krait venom used was of a

strength of 0·0005 grm. in 1 cub. centim. Equivalents to 10 M.L.D. were mixed with antivenomous serum for 30 minutes before injection into animals. Rats were used and the M.L.D. of Cobra venom taken was 0·0005 grm. per kilo. and of Krait venom 0·0009 grm. per kilo.

Krait venom experiments.				Cobra venom experiments.			
Weight of rat in kilos.	Venom per kilo.	Serum per kilo.	Results.	Weight of rat in kilos.	Venom per kilo.	Serum per kilo.	Results.
	grm.	cub. centims.			grm.	cub. centims.	
0·108	0·009	2	Dead in 4 hours.	0·183	0·005	2	Dead in 6 hours.
0·129	0·009	3	Dead in 2½ "	0·175	0·005	3	Dead in 45 "
0·120	0·009	4	Dead in 1½ "	0·188	0·005	4	Dead in 45 "
0·112	0·009	5	Dead in 2½ "	0·166	0·005	5	Remained well.
				0·087	0·005	nil	Dead in 1½ hours.
					(Control.)		

CALMETTE'S antivenomous serum therefore, in the amounts effectual against Cobra venom, appears to be ineffectual against Krait venom.

The integrity of the endings of the phrenic, vagi and sciatic nerves was examined in the majority of animals which died, the various nerves being exposed just at death. Induction stimuli were applied, and invariably there was noted definite diminution in the response to such stimuli—fibrillary twitchings of the diaphragm, for example, occurring with a stimulus to the phrenics where in the normal animal a complete contraction of that muscle would occur; and the vagal stimulation required to stop the heart was almost invariably greater than that required in the case of normal animals, whilst in some even the maximal stimulus failed to do more than slow the heart. In the case of rabbit No. 15, which recovered, there was definite evidence of paralysis of the extremities. This was at first more noticeable in the fore limbs, and the paralysis seemed to pass off in the same order, the hind limbs being the last to recover.

The comparative effect on skeletal muscle and nerve of the action of the venom was investigated on the frog's gastrocnemius with the sciatic nerve attached. The sensitiveness to stimulation by a Faradic current was ascertained in the case of the nerve and the muscle respectively, and the nerve was then placed in a fresh solution of the Krait venom dissolved in 0·65 per cent. tap-water saline where it remained for 22 hours, the muscle being carefully kept from contact with the poison and placed in normal saline. The strength of the venom solution used was 1 : 10,000. At the end of 14 hours the excitability of the nerve was unimpaired and at the end of 22 hours was only very slightly impaired.

At the same time, with a similar nerve-muscle preparation, the muscle was placed

in the venom solution, and in 2 hours and 20 minutes it had become much less excitable, though the excitability of the nerve was unimpaired. At the end of $3\frac{3}{4}$ hours no response could be elicited by stimulating the nerve, and it required strong direct stimulation of the muscle to cause any contraction. After $5\frac{1}{4}$ hours no contraction of any part of the muscle occurred with the strongest direct stimulation. It will be observed therefore, that the muscle was gradually poisoned. The ends of the nerve within the muscle appeared to have been poisoned before the muscle had lost its power to contract and before the nerve trunk was appreciably affected.

In order to define more nearly the loss of excitability of the nerve and muscle a similar experiment was performed with more frequent observations. The same characteristics in the action were observed. After 2 hours the excitability of the nerve was unimpaired, but during the next 20 minutes it diminished rapidly and was entirely lost. The muscle had gradually become less responsive, and finally ceased to contract even on the strongest stimulation, 1 hour after the nerve ends had been poisoned and $3\frac{1}{2}$ hours after the muscle had been placed in the poison. Immediately after death the muscle was found when tested by litmus paper to be acid in reaction.* Our previous conclusions are therefore confirmed.

EXPERIMENTS to determine the Minimal Stimulation required to produce Muscular Contraction, measured by the Distance between the Secondary and Primary Coils, expressed in millimetres.

Time.	Poisoned muscle.	Protected nerve of poisoned muscle.	Poisoned nerve of protected muscle.
Before poisoning	230	390	390
hrs. m.			
0 30 after poisoning . . .	230	385	405
1 0 " " . . .	210	380	—
1 30 " " . . .	210	400	—
2 0 " " . . .	170	420	410
2 5 " " . . .	160	440	—
2 10 " " . . .	140	170	—
2 25 " " . . .	130	0	415
2 50 " " . . .	100	0	—
3 5 " " . . .	70	0	—
3 10 " " . . .	50	—	—
3 15 " " . . .	30	—	—
3 20 " " . . .	0	—	—
14 0 " " . . .	...	...	400
22 0 " " . . .	...	...	305

The intermediate superfluous readings are omitted.

* The indication given of a direct action on muscle structure by the early occurrence of an acid reaction was pointed out by FRASER, "On the Kombé Arrow Poison (*Strophanthus hispidus*)," 'Roy. Soc. Edin. Soc.', 1869-70, p. 102.

The blood was examined in several cases immediately after death and in no case was any appearance of ante-mortem clotting found. Blood films from blood taken from the left side of the heart of rats immediately after death following a lethal dose of Krait venom showed, on examination of the red blood corpuscles, no evidence of hæmolysis having occurred. The corpuscles showed no abnormality, and serum separating spontaneously from blood in capillary tubes showed no hæmoglobin staining. The blood even from the left side of the heart was extremely venous in appearance.

Action on Blood Vessels.

One of us has shown that Cobra venom produces a marked constriction of the blood vessels of the frog when perfused through them in very dilute solution, the limit of this being reached at a strength of 1 : 10,000,000. It is of interest, therefore, to compare a similar action which we have found to be produced by Krait venom.

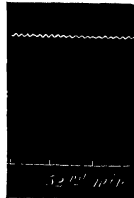
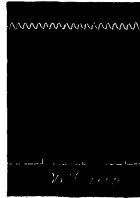
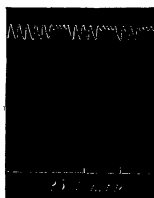
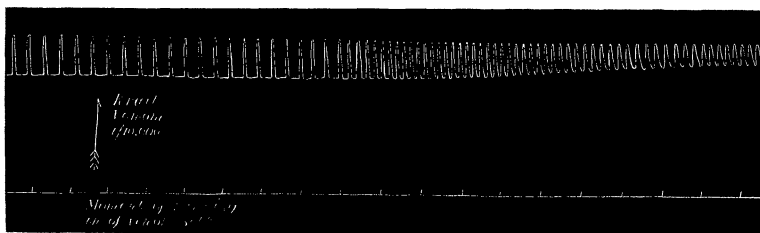
The brain and spinal cord of a frog having been carefully destroyed from half an hour to an hour before the beginning of the experiment, the thorax was opened and a cannula placed in one aorta (the other being ligatured). The venæ cavæ were cut and the cannula was attached by glass tubes to a series of MARRIOTTE'S flasks, by which the level of the fluids to be perfused was maintained at a constant height of 17 centims. The normal calibre of the vessels was ascertained by causing 0.65 per cent. sodium chloride solution to flow through them for from 10–20 minutes until the flow was uniform, the outflow being collected in graduated measures and the amount noted every 60 seconds. The venom dissolved in the same strength of saline solution was then perfused for from one hour to one hour and a-half. We had, unfortunately, too small a quantity of the venom to define the limits of its action with accuracy, but our experiments show that it produced an effect similar to that of Cobra venom but very much less in degree. In the strength of 1 : 500,000 we were unable to find any certain evidence of constriction, but in the strength of 1 : 250,000 the flow diminished to $\frac{1}{2}$ in 23 minutes and to $\frac{1}{4}$ in 69 minutes. There was no recovery when the normal saline was again perfused. With a strength of 1 : 100,000 the flow diminished to $\frac{1}{2}$ in 35 minutes, and with a strength of 1 : 50,000 the flow diminished to $\frac{1}{3}$ in 38 minutes.

Perfusion of the Frog-ventricle with Krait Venom.

This was carried out in a modification of SCHÄFER'S plethysmograph. Blood mixture was used for the normal, and as a vehicle for the venom. It consisted of 1 part of ox blood diluted with two parts of RUSCH'S modification of RINGER'S fluid. *Rana esculenta* was the animal used to provide the heart.

Experiment 1.—A frog-ventricle was perfused with a solution of Krait venom of a strength of 1 : 10,000. The following table gives the rate and the amplitude of beats in successive minutes :—

Minutes before venom solution was run in.	Number of beats per minute.	Amplitude of excursus of beat.	Minutes after venom solution was run in.	Number of beats per minute.	Amplitude of excursus of beat.
7	9.5	8.25	1	10	8
6	10.25	8.25	2	15	8
5	9.0	8.25	3	21.5	7.5
4	9.0	8.25	4	17.5	6.5
3	10.25	8.25	5	19.5	4.5
2	9.0	8.25	6	17.5	4.5
1	9.0	8.25	7	18	4.25
			20	18	3.25
			30	20	2.0
			40	25	1.0
			50	28.5	0.3



29.1.04. Perfusion of Frog-heart with Krait Venom Solution, 1 : 10,000. Read from left to right ; systole upwards ; time = 15 seconds. The minutes marked date from the running in of venom. The extracts are from a continuous tracing.

Experiment 2.—A frog-ventricle was perfused with a solution of Krait venom of a strength of 1 : 100,000. The tracing was continued for 52 minutes after the venom perfusion began. The result was entirely negative.

One of us* has shown that Cobra venom acts powerfully on the frog-ventricle, killing it in systole, when used in strong solutions, and acting as a tonic and stimulant

* Captain R. H. ELLIOT, "A Contribution to the Pharmacology of Cobra Venom;" communicated to the Royal Society, January, 1904; abstract at 'Roy. Soc. Proc.,' vol. 73, p. 183.

when the strength of the venom was weaker. Thus a 1 : 100,000 solution of Cobra venom killed the frog-ventricle in 20 minutes, in a position of tight systole, whilst a 1 : 10,000,000 solution distinctly quickened the beat. We have here a similar though far weaker action.

It would have been desirable to test the action of stronger solutions of the venom. This we were prevented from doing by the smallness of our stock of venom.

Kymographic Experiments.

Experiment 1.—A rabbit, weighing 3·75 kilos., was etherised; blood pressure was taken by the carotid, and respiratory movement was registered by means of a double stethograph. 0·004 grm. of Krait venom was dissolved in 1 cub. centim. of RINGER's fluid and injected into the left external jugular vein, the injection lasting 40 seconds. The blood pressure before the injection registered 126 millims. of mercury; 1 minute after injection it had fallen to 92; it then recovered steadily and stood at 116 millims. in the 5th minute after injection. Meanwhile respiration had been getting slower and shallower, and practically ceased in the 8th minute. An asphyxial rise of blood pressure then took place, a rise to 128 millims. being registered. A drop in pressure followed, the level reaching 82 millims. in the 10th minute. There was no evidence of cardiac inhibition on the blood-pressure tracing to explain the early fall, and it was only when asphyxiation was well marked that the heart-beat slowed. Under artificial respiration the blood pressure rose, and attained its former high level. When the artificial respiration was temporarily stopped, at intervals, a steep rise of pressure invariably occurred; a resumption of artificial respiration brought the pressure back to its former level again at once. Slowly and steadily the pressure fell, though the heart-beat remained comparatively rapid. After 1 hour 20 minutes of artificial respiration the experiment was stopped. In the meantime the stoppage at intervals of the artificial respiration had clearly shown that no respiratory effort whatever was being made by the animal. Whilst it was still under the anæsthetic the chest was opened and the phrenic, vagal, and sciatic nerves were tested with the secondary current. Neither the phrenic nor sciatic nerves responded to even maximal stimuli by so much as the production of a muscular twitch. Maximal stimulation of the vagi slowed, but did not stop the heart.

Since the M.L.D. of Krait venom for rabbits is 0·00008 grm. per kilo., this animal received over thirteen times the subcutaneous M.L.D. intravenously.

Experiment 2.—A dog, weighing 9 kilos., received an intravenous injection of 0·005 grm. of Krait venom dissolved in 5 cub. centims. of RINGER's fluid. The tracings in order from above are: (1) Intestinal volume recorded by a plethysmograph (SCHÄFER's); (2) blood pressure taken in the carotid; (3) respirations recorded by a double stethograph; (4) 10 seconds time-marks; (5) signal. The injection was made by the left external jugular, and took 72 seconds. There was an immediate fall of blood pressure, accompanied at first by a fall and later by a distinct rise in intestinal volume. There is no slowing of the beat, so we apparently have not got cardiac inhibition to deal with. The rise in intestinal volume will explain the fall in general blood pressure, if we may suppose that the first fall in intestinal volume (a matter of 20 seconds only) was due to a dilatation in another portion of the splanchnic area which had not yet reached the loop of gut under observation. Another possible explanation will be given presently. An extract from this portion of the tracing is here shown. The presence of Traube-Hering curves in the blood-pressure tracing supports the view that we have to do with a central vaso-motor dilatation. This dilatation continued throughout the experiment, and was increased when a second injection of 0·005 grm. of the venom was given 30 minutes after the first injection. In spite of this the general blood pressure remained high throughout the experiment. This is apparently to be explained

by the direct constriction of the systemic arterioles by the circulating venom. We have already dealt with this action.

Extracts from the tracing are given on pp. 337 and 338.

The accompanying table gives a short analysis of the tracing. Respiration was first excited and then gradually paralysed, just as occurs in Cobra poisoning. The experiment was stopped after 55 minutes, and the nerve-ends were tested by the secondary coil. The phrenics responded with the coil at 300 millims. from the primary coil, the sciatics at 280 millims., and vagal inhibition was got at 120 millims. The two first were, therefore, distinctly blunted in sensitivity; in the last this is doubtful:—

Time in minutes.	Number of heart-beats in 10 seconds.	Number of respirations in 10 seconds.	Amplitude of respiratory movement as shown by stethograph.	Blood pressure in millimetres of mercury.
1 before first injection .	18	11	millims. 10	140
1 after commencement of first injection . .	19	12	9	118
2 ditto	21	19	18	105
5 ditto*	16	11	6	136
10 ditto*	13	9	11	155
15 ditto*	14	11	4	164
20 ditto	20	10	21	162
25 ditto	17	9	9	168
30 ditto	17	9	3	151
A farther injection of 0·005 grm. of Krait venom was now given, in the same way as the last dose.				
31 ditto	17	8·5	3	148
32 ditto	18	9	4	144
35 ditto	19	7	2·5	167
40 ditto	19	7	1·75	162
45 ditto	19	6·75	3	164
50 ditto	20	6	2	174
55 ditto	20	5	3·5	172

It is possible that the anæsthetic was to blame in these three readings.

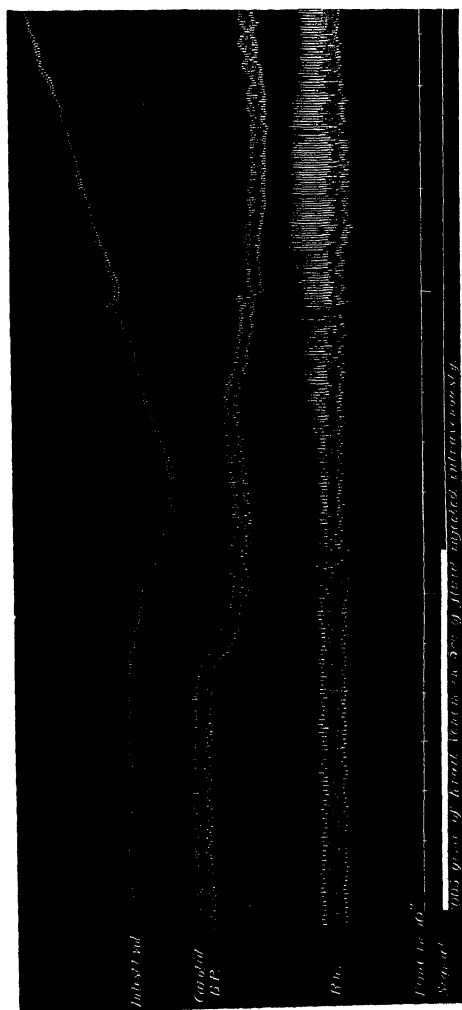
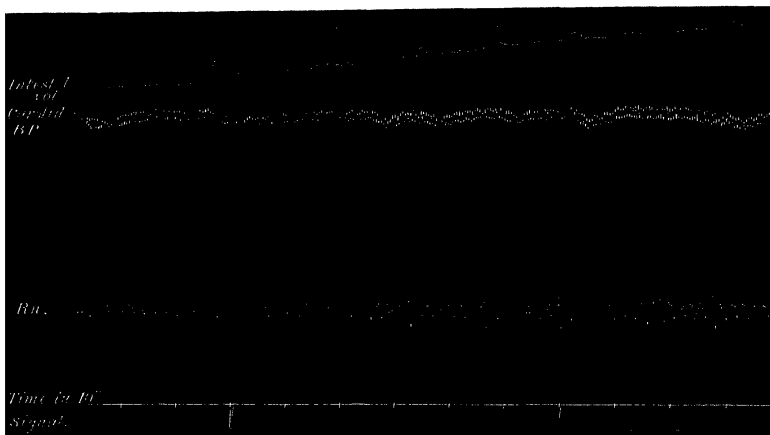


Fig. 2. Extract from Kymographic Tracing of Experiment 2. Commencement of experiment. Notice (1) fall in H.P.; (2) rise in intestinal volume; and (3) changes in respiration.

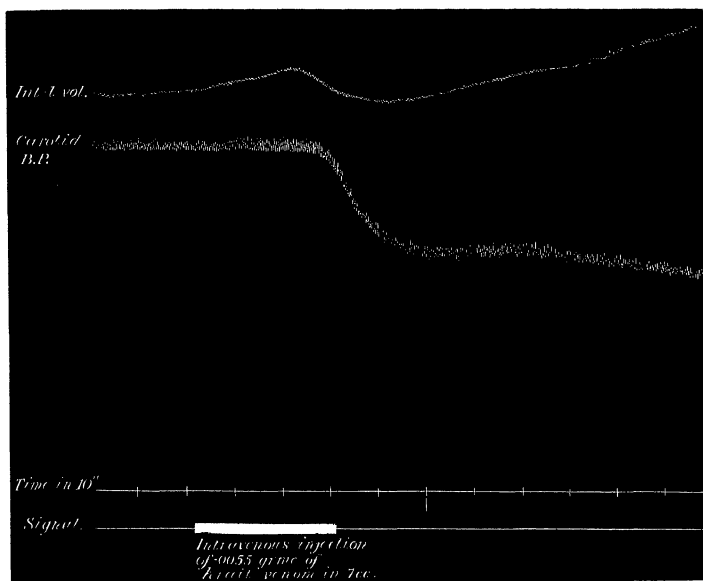


Extract from Kymographic Tracing of Experiment 2. The end of the tracing just before the experiment was stopped. Notice (1) the high B.P.; (2) the rising intestinal volume (the lever had been lowered several times); and (3) the irregular respiration.

Experiment 3.—The previous experiment was repeated on a dog weighing 5·5 kilos. The dose of venom injected was 0·0055 grm. (Krait venom), dissolved in 7 cub. centims. of RINGER's fluid. There was a speedy fall of blood pressure. Slightly preceding this there was a rise in the plethysmographic leve denoting a rise in intestinal volume; then followed a passive fall of intestinal volume, and immediately afterwards a great rise thereof. This rise in intestinal volume continued during the 17 minutes the tracing was taken, but the blood pressure recovered in spite of it. The following table gives the result recorded:—

Time in minutes.	Number of heart-beats.	Blood pressure in millimetres of mercury.
Before injection	26	128
1 after	26	86
2 "	26	79
3 "	27	73
4 "	27	75
5 "	27	76
10 "	25	94
15 "	23	108

An extract from the tracing is furnished on p. 339.



Extract from Kymographic Tracing of Experiment 3.

Experiment 4.—A rabbit, weighing 5 kilos., was etherised; blood pressure was taken in a carotid artery, respiratory movement was recorded by means of a double stethograph, and injections of Krait venom were made through the marginal vein of one ear.

The first injection consisted of 0.0006 gram. of Krait venom dissolved in 1 cub. centim. of normal saline solution, and took 1 minute to pass in. The second was given 22 minutes later, and consisted of 0.001 gram. of Krait venom in 1 cub. centim. of normal saline solution; it took 25 seconds to pass in.

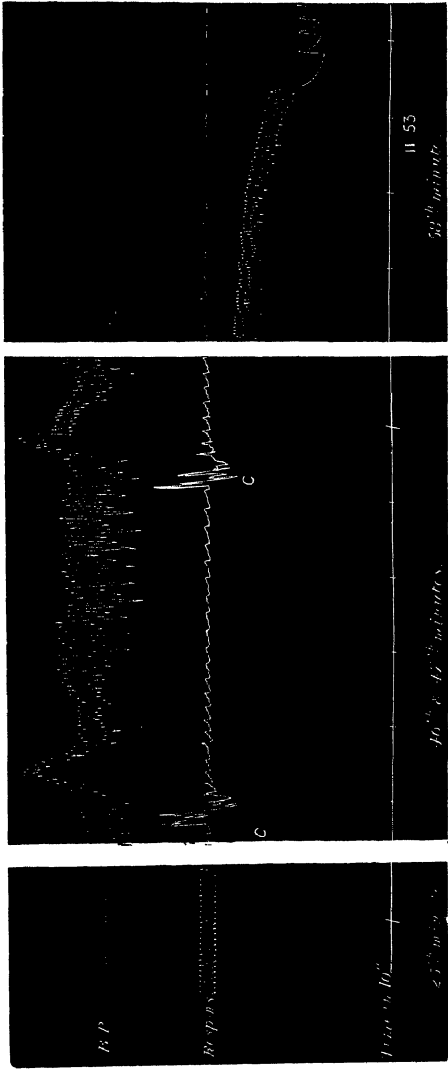
The following table shows the results obtained :—

Time in minutes.	Heart-beats in 10 seconds.	Respirations in 10 seconds.	Respiratory excursions.*	Blood pressure in millimetres of mercury.
			millims.	
Before injection	47	19	6	136
1 after ditto	43	14	5·5	132
2 ditto	43	14	5·25	129
3 ditto	44·5	13·5	5	126
4 ditto	44	14	5·5	124
5 ditto	44	14	5·5	122
6 ditto	42·5	15·5	5·25	124
10 ditto	43	17·5	5·5	126
15 ditto	41	17	4·5	121
20 ditto	39	15	4·75	119
22 ditto	40	16	5	121
The second injection was given just before the last reading was taken.				
23 after first injection	42	15	4·5	123
24 ditto	42·5	14·5	4·5	115
25 ditto	42	13·5	4	112
26 ditto	42	13	4·25	112
30 ditto	40	12	4·75	111
35 ditto	38	10·5	5	113
40 ditto	35	10	4·75	112
45 ditto	17	6·5	2	114
50 ditto	13	3	1	124
55 ditto	29	4·5	2	65
58 ditto	18	0	0	38
60 ditto	Dead	—	—	—

Experiment 5.—A rabbit, weighing 3·5 kilos., was prepared in all respects like that in the previous experiment, and received 0·002 grm. of Krait venom, dissolved in 1 cub. centim. of RINGER'S fluid injected into the marginal vein of one ear; the injection took 40 seconds. The result is shown in the following table :—

Time in minutes.	Heart-beats in 10 seconds.	Respirations in 10 seconds.	Respiratory excursions.*	Blood pressure in millimetres of mercury.
			millims.	
Before injection	53·5	24	2	80
1 after ditto	54	23	2	70
2 ditto	54	22	2	64
5 ditto	52	21	1·5	70
10 ditto	51	16	1	86
15 ditto	49	15·5	0·5	80
16 ditto	41	9	0·3	64
17 ditto	42	4·5	1	50
18 ditto	Dead	—	—	—

* *Note.*—The respiratory excursions given in Experiment 4 and 5 is the excursions in millimetres of the lever point of the double stethograph.



Extracts from Kymographic Tracing of Experiment 4.

*At end of injection**In 10th minute**At end of experiment*

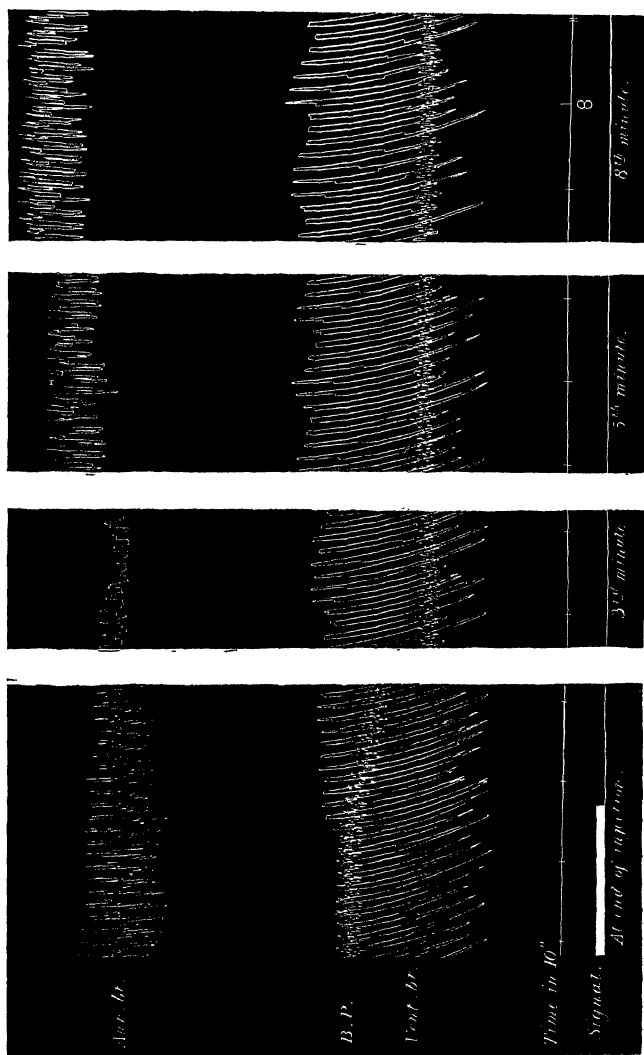
Extracts from Kymographic Tracing of Experiment 5.

Experiment 6.—A cat, weighing 2·2 kilos., was etherised; blood pressure was taken in a carotid artery; the chest was opened and the movements of the auricle and ventricle were recorded by means of levers attached to their walls by silk threads; artificial respiration was maintained throughout; the injection was made through an external jugular vein, time was registered in 10 seconds.

After obtaining a normal, 0·002 grm. of Krait venom was injected, in 1 cub. centim. of RINGER'S fluid; the injection took 18 seconds. There was an immediate fall of blood pressure, and at the same time there was clear evidence of a weakening of the auricular beat, accompanied by some slowing of the heart. The ventricle did not appear to be affected. The force of the auricular beat recovered rapidly, was normal in the fifth minute, and during the next 4 minutes was decidedly in excess of what it had been before the injection. Extracts from the tracing are given; the following table shows the changes that occurred in heart-rate and blood pressure:—

Time in minutes.	Number of heart-beats.	Blood pressure in millimetres of mercury.
Before injection	27	121
1 after ditto	26	106
2 ditto	22	87
3 ditto	20	86
4 ditto	21	88
5 ditto	21	88
6 ditto	21	89
7 ditto	20	90
8 ditto	20	90
9 ditto	20	90

Experiment 7.—The previous experiment was repeated on a cat weighing 2 kilos., a dose of 0·003 grm. of Krait venom being injected. The results yielded bore out those of the previous experiment, and need not therefore be given in detail.



Extracts from Kymographic Tracing of Experiment 6.

Summary of Conclusions.

We are now in a position to consider the very diverse influences which act upon the blood pressure in Krait poisoning, and we find a very different state of affairs from that which holds in Cobraism.

(1) The heart-muscle, or its nerve-end mechanism, is acted on directly by Krait venom. This action is strictly comparable to that of Cobra venom, but is much less strong. The tracings, taken with the mammalian heart exposed and with levers attached to the auricular and ventricular walls, show, however, that an important increase of cardiac force is in this way brought about. A decided increase of heart rate was also observed in several tracings.

(2) There are indications of a feeble cardio-inhibitory action. This supervenes so rapidly, when it is seen at all, that it must almost certainly be of central origin. The action of this venom is thus again brought into line with that of Cobra poison, and is much more feeble than that of the latter in this particular phase of its activity. So much so indeed, that the inhibition is quickly and easily masked by the direct effect of the venom on the heart, the latter action is manifested more slowly. It is probable that inhibition of the heart is responsible for the early fall in blood pressure, which is sometimes noticed before the plethysmographic lever has recorded any change in intestinal volume. The slight extent of the inhibition, even when it is observed, may be judged from the fact that it is only the auricle which shows the change, the ventricle curve not being perceptibly affected in our tracings. Moreover slowing of the heart was never well marked and was often absent.

(3) The direct action of the venom on the arterial muscle, or on its nerve-end mechanism is, as we have shown above, such as to produce vaso-motor constriction. This effect can be clearly got with solutions of 1 : 250,000 and below, but we failed to get it at 1 : 500,000. It is therefore much weaker than the corresponding action of Cobra venom, which can be demonstrated in a solution of 1 : 10,000,000.

(4) The injection of Krait venom is followed by a very early and decided fall of blood pressure. This fall has been shown to be mainly due to a dilatation of the vessels of the splanchnic area, and is almost certainly central in origin.

It is obvious that the direct tonic effect of Krait venom on the heart and vessels tends to raise the general blood pressure. On the other hand, the slight cardio-inhibition and the powerful action on the central vaso-motor (leading to dilatation of the splanchnic area vessels), are responsible for the marked early fall so constantly met with. The two latter forces come into action before their opponents have time to manifest themselves.

There is yet another factor to be taken into account, viz., the influence of the poison on the respiratory mechanism. We regard this as the most important element in the whole case.

Our experiments have shown that death in Krait poisoning is due to failure of the respiratory mechanism and, moreover, that when this failure occurs, the phrenic and other motor nerve-ends are still active, though their sensitivity is always more or less impaired, and often very decidedly so. We, therefore, conclude that the interference with respiration, which culminates in the death of the subject, is due to a direct action of the venom on the respiratory centre. This view is borne out by the rapidity with which respiration is often affected in these experiments. The limited quantity of poison at our disposal prevented us from pushing this question farther, as we would like to have done. We hope at a later date to apply Krait venom directly to the exposed medulla oblongata with a view to ascertain whether the centre can be directly poisoned in this way without the nerve-ends being at all affected. One of us has shown that such is the case with Cobra venom. We are, however, distinctly of the opinion that the blunting of nerve-end sensibility, which we have observed, is sufficient to add materially to the embarrassment of an already damaged respiratory centre.

Respiratory failure, slowly developed, brings in its train all the phenomena of slow asphyxiation. The circulation of venous blood through the medulla oblongata tends to slow the heart by acting on the vagal cardio-inhibitory centre. It must also stimulate the vaso-motor centre, and so tend to cause arteriolar constriction, and a consequent rise in blood pressure. These two latter forces will, of course, act in opposition to each other, whilst both are superadded to those which we have already discussed.

It is, therefore, very evident that the problems which lie hidden in a blood-pressure tracing taken from a Krait poisoned animal are very complex. The main features are, however, obvious enough. The early fall of pressure is due to a central vaso-motor action on the splanchnic area, assisted and sometimes probably preceded by a small measure of cardio-inhibition of central origin. The recovery of pressure which follows is attributable (1) to an increase in cardiac force brought about by the direct action of the venom on the heart, and (2) to the direct constrictive action of the venom on the vessel walls. Later, as respiration progressively fails, the asphyxiation of the medulla oblongata is thrown into the scale in favour of vaso-constriction. Nevertheless, the direct action of the venom on the vaso-motor centre is so powerful that one never sees the sustained high levels and the final great rises of blood pressure which are a common feature of a tracing in Cobra poison experiments.

Most of the kymographic work of this paper has been carried out in the Physiological Laboratory of Edinburgh University. For the permission to use his apparatus, and for his valuable advice and assistance, we are deeply indebted to Professor E. A. SCHÄFER.

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XII. *Further Note on the Remains of Elephas Cypriotes from a Cave-Deposit in Cyprus.*

By DOROTHEA M. A. BATE.

Communicated by Dr. HENRY WOODWARD, F.R.S.

Received April 18,—Read June 9, 1904.

[PLATES 21 AND 22.]

PREVIOUS to 1901, no systematic search of the cave deposits of Cyprus appears to have been attempted. The geology was studied by M. ALBERT GAUDRY, who published in 1862 an elaborate work with a geological map,* and Drs. UNGER and KORSCHY, in 1865,† also gave a geological map of the island, differing somewhat from that of their predecessor.

As long ago as 1700 the Dutch traveller CORNEILLE LE BRUN‡ (VAN BRUYN), published an account of his wanderings in Cyprus and the Levant, and mentions having visited a bed of bones, believed to be those of saints, not far from the Monastery of Haghios Chrysostomos. A drawing of one of these bones is given which Dr. FORSYTH MAJOR has since shown to be that of *Hippopotamus minutus*.§

The author started in 1901, in expectation of discovering an extinct fauna in this ossiferous breccia, and this expectation was amply fulfilled, and altogether no fewer than twelve ossiferous cave-deposits were found, five at Cape Pyla in the south-east, and seven on the southern slopes of the Kerynia Hills in the north of the island.

Two caves at Cape Pyla (mentioned by General DI CESNOLA in 1877, as containing human fossilised bones), were first visited. The rock in which these caves were found is a Miocene (probably Helvetian) limestone, weathered to a very great extent, and full of marine shells and corals as well as numerous Echinoids (*Clypeaster portentosus*), also met with in the Miocene limestones of Malta.

Here a number of caves were discovered in the cliffs, five of which yielded remains of *H. minutus*. These were: (1) The Red Cliff Cave; (2) the Great Anonymous Cave; (3) the Small Anonymous Cave; (4) Haghios Jannos; and (5) Haghios Saronda.

* 'Géologie de l'Île de Chypre,' Paris, 1862.

† 'Die Insel Cypern,' Wien, 1865.

‡ 'Voyage au Levant,' etc., Delft, 1700, p. 375.

§ 'Zool. Soc. Proc.,' vol. 2, 1802, p. 107.

This last is the cave to which formerly pilgrimages were made, and candles burned in honour of the supposed sacred remains of saints.

The rocks of the Kerynia Hills are of uncertain geological age, no fossils having been obtained from the limestone of which they are chiefly composed. Professor GAUDRY concludes that the rock is of Cretaceous age and, therefore, the oldest sedimentary deposit in the island.

The seven caves discovered in this range are all on the southern side between the Aghirdhir Pass and the village of Kythraa, in a low broken line of cliffs, which runs parallel with the main ridge. They are named: (1) Coutzaventis; (2) Haghiōs Chrysostomos; (3) Anoyero Spelios; (4) Dikomo Mandra; (5) Haghiōs Elias; (6) the Elephant Deposit; and (7) the Western Cave.

Most of these caves have, by reason of long atmospheric erosion, partially or wholly disappeared, leaving the stalagmatic flooring containing mammalian remains unprotected and exposed, often at a considerable distance from the face of the cliffs. But although many of them are now almost obliterated by the falling in of the roof and walls, it was found that wherever this has happened the limits of the floor are sharply defined by the hard ossiferous deposit and the stalagmitic floor. In close proximity there are caves still preserved containing precisely similar deposits.

The fauna of the caves is comparatively scanty, the only other important extinct form besides the dwarf elephant and hippopotamus being a new species of Genet (*Genetta plesictoides*),*

The remains of the pigmy elephant, *Elephas cypriotes*, from Cyprus, with regard to which a short note has already been published,† were all procured from a single cave deposit situated on the southern side of the Kerynia Range. This restriction is somewhat remarkable in view of the fact that there are several otherwise similar deposits in the immediate vicinity, one small cave being only about 150 yards distant. The teeth and mandibular rami of this species were present only in small numbers but were associated with a large quantity of remains of *H. minutus*. This deposit, like others found in the same locality, was evidently the floor of a former cave of which but one original wall is still standing.

During the excavations made here many bones and teeth were found fractured; and that this had taken place subsequent to their accumulation on the floor of the cave was proved by the parts of a bone or tooth being found in natural juxtaposition, only falling apart when removed from the surrounding earth or rock. The opposing surfaces thus revealed were brown and discoloured by the infiltration of water and earth, showing that the fractures had not taken place at any very recent date. This occurred similarly in all the deposits where the cave walls and roof have disappeared, but amongst the remains of *H. minutus* obtained from a large cave in the same hills, none were found to have been broken prior to their separation from the matrix.

* 'Zool. Soc. Proc.', 1903, vol. 2, p. 121.

† 'Roy. Soc. Proc.', vol. 71, 1903, pp. 498-500.

The collection of elephant remains includes forty-six molars, or parts of molars, also a few isolated "tooth plates"; amongst the former are a number of perfect teeth, some still retaining their position in the jaws. In several instances pairs of molars similar in every respect, except in belonging to opposite sides of the mouth, are thought to have undoubtedly belonged to a single animal. Besides these one perfect and six slightly damaged specimens of permanent incisors and fragments of others were obtained. The only portion of the skeleton found was the imperfect distal extremity of a femur.

Incisors.

Milk Incisors.—Unfortunately none of the small milk incisors were found: it would have been of much interest to discover whether these were, or not, capped with enamel like those of the African and Maltese species.*

Permanent Incisors.—Several good pieces of tusks procured probably include those of both the male and female elephants and all differ from examples from Malta in being considerably compressed laterally (Plate 22, fig. 9), more especially those of the female and young. They appear to be curved to a greater extent than is the case in those of *E. melitensis*, and are slightly more so than the tusk in the collection of the British Museum ascribed by BUSK to *E. falconeri* and figured in the 'Transactions of the Zoological Society.'† The Cypriote specimens resemble the Maltese in the ivory being longitudinally grooved; this feature is quite noticeable in the young teeth, but generally becomes almost lost exteriorly in aged examples, though it is still very apparent on the inner layers of the ivory when the outermost has been removed. The one exception to this is found in the incisor (B.M., m. 8610), believed to be that of an adult female, in which I have been able to find no trace of this longitudinal channelling.

The central portion of a tusk of *E. melitensis* in the collection of the British Museum (No 44,322) measures 157 millims. ($6\frac{1}{4}$ inches) in circumference, which size is not equalled in any of the specimens from Cyprus ('Trans. Zool. Soc.' vol. 9, Plate 11, fig. 13). The largest (B.M., m. 8614, Plate 22, fig. 8), includes almost the entire tooth except the proximal end and the extreme tip; it measures 297 millims. (12 inches) along the outer curve, its greatest circumference being 134 millims. Another piece (B.M., m. 8610) containing part of the pulp cavity, is 279 millims. (11 inches) long, and has a girth similar to the last-mentioned tooth. Both are yellowish-brown in colour and exceedingly brittle. Almost the whole of a further specimen (B.M., m. 8609) was secured, although in several pieces, and is considered to be that of a young male. The tip is somewhat worn on its upper surface, while the under surface for a short distance is excessively so, the centre of the tooth

* See FALCONER, 'Pal. Mem.' vol. 2, Plate 11, fig. 3.

† Vol. 6, Plate 52, fig. 48.

being exposed at this point. Another (B.M., m. 8609A), the distal extremity of which is missing, is evidently the tooth of a slightly older animal than the owner of the last mentioned. Of the two remaining tusks, one (B.M., m. 8610) I believe to be that of an adult, and the other (B.M., m. 8608, Plate 22, fig. 7), that of a young female; as may be seen from the following table of measurements they are both much shorter in comparison with their bulk than are the other specimens, and are also considerably more compressed laterally. The distal end of the latter is curiously worn round the whole of its circumference, the layers of ivory becoming lost as they approach the tip, the outer one disappearing at a distance of about 39 millims. from the point of the tooth.

The following table shows some measurements, given in millimetres, of the best preserved specimens of tusks obtained.

	(M. 8610.*)	(M. 8608.)	(M. 8614.)	(M. 8610.)	(M. 8609.)	(M. 8609A.)
Length along outer curve . . .	227	133	297	279	186	215
" " inner " . . .	158	116	234	208	170	202
Greatest circumference . . .	123	85	134	134	85	94
Greatest transverse diameter	28	23	37	34.5	21.5	25

Upper Molars.

Anterior Milk Molar (m.m. 2).—No specimens of the anterior milk molar (m.m. 2) were obtained, although the alveolus of this tooth is still to be seen in a portion of a left maxilla (B.M., m. 8617, Plate 22, fig. 3), which also contains a second milk molar (m.m. 3). In all probability this tooth resembled that of the Maltese species in being composed of three plates. The absence of a specimen for comparison with that of the other dwarf forms is all the more unfortunate on account of the examples from Malta being peculiar in having but a single conical root. Judging from the alveolus the same was the case in the present instance.

Penultimate Milk Molar (m.m. 3).—This milk molar is represented by two worn examples, one of these being still attached to a small portion of the left maxilla, and in a perfect state of preservation. This last (B.M., m. 8617) consists of five ridges, all worn, and a small posterior talon. Possibly there was also a further small anterior ridge, though this cannot be accurately ascertained on account of the advanced state of wear of both specimens. In the figured specimen (B.M., m. 8617, Plate 22, fig. 3), the four posterior plates of dentine are very little thicker than the intervening areas of cement; its greatest length is 39 millims. and its breadth 22 millims.

There are often both broad and narrow-crowned specimens of the different molars; the second milk molars obtained are of the former variety, and curiously enough the

* The measurements of this tusk are only approximate, it being still partially embedded in the matrix.

breadth of their crowns is not equalled by that of the single specimen obtained of the following upper m.m. 4 (B.M., m. 8603). It is also noteworthy that the above-mentioned specimens are considerably larger than the representatives of this milk molar from Malta, whereas, amongst the other teeth in the series, those from Cyprus are almost invariably smaller than, or barely attain, the size of the corresponding molars of *E. melitensis*.

Last Milk Molar (m.m. 4).—Only one tooth in the collection (B.M., m. 8603, Plate 22, figs. 4 and 4A) undoubtedly belongs to this position in the series. It is perfectly preserved and consists of seven plates, a small anterior ridge and a talon; the stout anterior fang is still present, and the fourth plate just coming into wear. The antero-posterior length of the tooth is 47 millims., and its height 34 millims., whilst the crown is of medium width, being 18·5 millims. at its widest.

First True Molar (m. 1).—It is often a matter of some difficulty to decide whether a tooth should rather be considered as a last milk molar or a first true molar. The two generally consisting of a similar number of plates, they must necessarily be chiefly distinguished by the difference in size, which is usually considerable, though, as this varies, large specimens of the milk tooth may approach very closely to the smallest examples of the succeeding molar. One seemingly constant characteristic in the first upper true molars is the very much greater height of their crowns compared with that of the last of the upper milk series. This difference is well shown by the specimens of these two molars figured in Plate 21, fig. 4A, and Plate 22, fig. 4A. The first true molar (B.M., m. 8602, Plate 21, figs. 4 and 4A) is a partially worn tooth somewhat damaged posteriorly. Its greatest height is 53·5 millims.; its antero-posterior length 49 millims., and the width of the crown 25 millims.; the number of plates of which it is composed is not accurately determinable, but was probably either seven or eight.

Second True Molar (m. 2).—This molar is represented by three specimens, the crowns of two of these being extensively worn, whilst that of the third (B.M., m. 8601) is but slightly invaded. Two views of one of the former (B.M., m. 8599) are given in Plate 22 (figs. 1 and 1A). The number of plates in this molar appears to vary from eight to ten.

Third True Molar (m. 3).—Three isolated molars in the collection are considered to be the last of the upper series. Two of these (B.M., m. 8591 and B.M., m. 8601) from their similar appearance are probably those of the same animal; both are tall and extremely narrow, in this respect resembling the lower molar, B.M., m. 8591 (Plate 22, figs. 6 and 6A), which might almost have been taken for one of the opposing teeth were it not that they differ somewhat in the state of wear of the crowns. The anterior ridge in each is almost entirely worn away, probably from pressure against the preceding teeth, for the fourth plate only just coming into use shows that these could not have progressed far along the alveolar cavity. The greatest width of the abraded surfaces of the crowns is only 25 millims., whilst the

height, taken behind the anterior fang, which is broken off in both cases, is 67 millims., and the total length 95 millims. in M. 8591 and 94 millims. in M. 8601, though these last measurements would have been slightly greater were the teeth quite perfect.

The third, a half-worn tooth (B.M. M. 8600, Plate 22, figs. 2 and 2A), is more robust than the two former specimens, its broader crown being 30·5 millims. across, and its greatest height 71 millims. The first plate has been worn away, and there is one, or possibly more, missing posteriorly. When compared with the last upper molars of *E. melitensis*, this, the largest of the three examples from Cyprus, is found to be appreciably smaller, though the pattern of its crown is somewhat similar to that of a tooth figured by Dr. FALCONER ('Pal. Mem.', vol. 2, Plate 11, figs. 1 and 1A). However, this last is larger, its greatest height being 84 millims., and the breadth of the crown 37 millims.; these measurements in the Cypriote specimen being, as already mentioned, respectively 71 millims. and 30·5 millims. Larger still, both in height and width, are a pair of last molars of this Maltese species situated in a portion of the upper jaw, and differing further in showing a slight central expansion of the plates of dentine.*

Lower Molars.

In the lower molars the plates before, and at the time of, coalescing, lie in a slanting position, sloping downwards and backwards from their upper aspect. The anterior plates very soon assume a vertical position, and later incline forwards, so that the lower edges are well in advance of their worn surfaces, thus reversing their original direction. As the tooth advances and the crown becomes more worn, a similar alteration is carried out in all the successive ridges. This also takes place in much the same manner in the molars of the upper jaw.

Anterior Milk Molar (m.m. 2).—As already mentioned, not a single specimen of this tooth was obtained.

Penultimate Milk Molar (m.m. 3).—A perfectly preserved worn example of this molar was obtained (B.M., M. 8597, Plate 21, fig. 5), besides which an unworn tooth (B.M., M. 8623), consisting of four plates, ought, most probably, to be referred to this position in the series. Like the corresponding teeth of the upper jaw already described, the crown of the former is wide, being 18 millims. across, while its antero-posterior length is 35 millims. An undamaged specimen of this milk molar of *E. melitensis* (B.M., 44,226) in the British Museum collection† is 35 millims. in length, and only 15 millims. wide.

Last Milk Molar (m.m. 4).—This tooth is represented in the collection by six well-preserved specimens, as well as by several others more doubtfully referred to this position in the series. The almost entire left mandibular ramus of a young individual

* 'Zool. Soc. Trans.,' vol. 9, Plate 4, fig. 1.

† Figured 'Zool. Soc. Trans.,' vol. 9, Plate 1, figs. 8 and 8A.

(B.M., M. 8595, Plate 22, fig. 5A) was obtained; anteriorly this is somewhat elongated, being 45 millims. from the front of the symphysis to the edge of the half-worn tooth it contains. There is a single foramen situated below and in front of the molar, but this number, or position, is not constant, for in more than one jaw-bone in the collection there are two of these openings present.

A drawing is given (Plate 22, fig. 5) showing the crown view of the tooth contained in the ramus just noticed (B.M., M. 8595). It consists of eight plates, the sixth of which, very slightly abraded, is only just coming into wear, and at its hinder end is a well-defined talon. Its total antero-posterior length is 56 millims., its height, measuring from the edge of the fourth plate, 37 millims., while the greatest width of the crown is 17.5 millims.

Like the other worn examples of the last milk molar, this tooth is strongly convex inwards, the plates consequently running in an oblique direction; this seems more strongly marked than in the corresponding teeth from Malta. The three anterior ridges are much worn, but there is no simultaneous mesial expansion of the plates of dentine and their surrounding bands of enamel such as occurs in many of the similarly worn molars of *E. melitensis*. The intervening spaces filled with cement are narrow, which is often the case in teeth in an advanced state of wear. Dr. LEITH ADAMS has shown* that this diminution of the areas of cement may be due to their rapidly decreasing in thickness from the top of the tooth downwards. However, that the cement does not always so decrease in the teeth of the Cypriote species is shown in the drawing of a section (B.M., M. 8605, text-fig. 1) in which it maintains an almost uniform thickness until an extremely low level is reached. This figure is interesting when compared with that given by Dr. LEITH ADAMS,† from which it differs markedly. The second section of a specimen from Cyprus (B.M., M. 8618, Plate 21, fig. 6) more closely resembles the one from Malta, and it seems probable that the width and extent of the cement areas is not constant in the molars of either the Maltese or Cypriote species, though it is more usual to find those of the latter nearly equalling in width the plates of dentine.

Two isolated last molars of the lower milk series (B.M., M. 8604) probably belonged to one individual, for they agree very closely in general appearance, size, and stage of wear; in both the last plate is just coming into use. They still retain their roots, which are long, the teeth being about 45 millims. in height; the crowns are broad, measuring 20 millims. from edge to edge at their widest. They

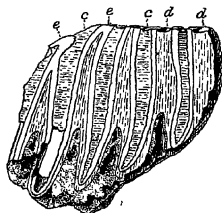


Fig 1.—Section of upper molar. B.M., M. 8605 (nat. size), c, cement; d, dentine; e, enamel.

* 'Zool. Soc. Trans.,' vol. 9, p. 6.

† *Op. cit.*, Plate 2, fig. 6.

show a slight indentation on the talons, which, considering their worn condition, may have been caused by pressure from the succeeding teeth pushing up from behind. Apparently two further examples, B.M., m. 8604 and B.M., m. 8621, likewise belonged to a single animal; in these the fangs have almost completely disappeared, thus materially altering the height of the teeth, which in this instance is only about 30 millims., showing a difference of 15 millims. from Nos. m. 8604 and m. 8621 just described. Compared with the above a corresponding molar of *E. melitensis* (B.M., 49,239) is slightly narrower in the crown, though its antero-posterior length is greater than that of the first described m.m. 4 from Cyprus (B.M., m. 8595), being 57.5 millims., although part of the front ridge is missing.

A worn tooth attached to an imperfect left mandibular ramus (B.M., m. 8597), and several unworn specimens showing an anterior flattening of the crown caused by pressure against their predecessors, are all referred to the third lower milk molar (m.m. 4).

First True Molar.—Several good specimens of this tooth are included in the collection. Two mandibular rami, each containing a first molar, are evidently those of the same animal; that of the right side (B.M., m. 8590, Plate 21, fig. 1A) is almost perfect, while that of the left (B.M., m. 8592) is considerably more damaged.

The tooth (B.M., m. 8590, Plate 21, figs. 1 and 1A) contained in the ramus just described, apparently consists of eight plates and is somewhat worn away anteriorly, although the enamel of the sixth plate is only slightly abraded and that of the seventh still concealed by its covering of cement. The crown is of medium width, measuring 22.5 millims. at its broadest. Considering the position and state of wear of this tooth, it might have been expected that the following one would be in a fairly advanced stage of growth; however, when the specimen was found, the cavity behind, which is separated by a septum of bone from the first molar, contained only a partially developed tooth plate 19 millims. wide and 14 millims. in height.

The specimen (B.M., m. 8596) shown in Plate 21, figs. 2 and 2A, is of special interest, being the only one in which the entire symphysis is preserved. The two anterior portions of the jaw are not known to have been found in connection, but it can hardly be doubted that they originally belonged to a single mandible. As indicated in the drawing they were discovered already broken off just behind the much-worn molar contained in each. It is a matter of some doubt whether these are first or second true molars, for, being considerably worn away anteriorly and only the five last ridges remaining, it is impossible to discover what was the original number of plates of which the teeth were composed. The greatest width of the crowns is 25 millims., almost equalling that of an example of the second lower molar (B.M., m. 8588, Plate 21, fig. 3), but from the size of this portion of the mandible I am inclined to believe that they are unusually broad-crowned examples of the first true molar. The areas of cement are wide and the ridges run very obliquely owing to the curvature of the teeth; these are much raised above the level of the alveolar border

and, viewed from the side, the plates are seen to be greatly reflexed. The beak is short and deflected, and the symphysis broad and of considerable antero-posterior length, measuring 32 millims. from its inner margin to the point of the beak. However these last particulars are not of much value, Dr. LEITH ADAMS having shown* to how great an extent individual differences occur in the anterior extremity of the mandible in the larger elephants.

A lower m. 1 of *E. melitensis* (B.M., 44,265) figured by Dr. LEITH ADAMS† agrees in its general proportions with the corresponding molars of *E. cypriotes* though differing somewhat in its crown pattern. This shows the persistence of the separation into islands of the dentine and surrounding enamel, this being more strongly marked than in specimens from Cyprus.

Second True Molar (m. 2).—A fine specimen was obtained of a right mandibular ramus (B.M., m. 8588, Plate 21, figs. 3 and 3A) which, though considerably larger, in shape resembles the example containing a first true molar already described (B.M., m. 8590). It contains a second true molar with all but the last two ridges in wear, while behind, and still separated from this by the bony septum, may be seen some of the germ plates of the succeeding tooth (Plate 21, figs. 3 and 3A). The front of this worn molar is missing; its present antero-posterior length is 86 millims., the height at its hinder end 57 millims., and the greatest width of the crown 23·5 millims. The inner half of the cement area connecting what are probably the third and fourth plates is hollowed, thus exposing the enamel of the latter to a depth of about 4·5 millims. and showing it to have a smooth and unlicated surface; the same is apparent in the enamel of the two teeth (B.M., m. 8596) figured in Plate 21, fig. 2.

Two other second lower molars, both of the left side, are among the collection, one (B.M., m. 8593) only partially worn, has a somewhat broad crown and apparently consisted of nine plates, though possibly there may have been an additional one posteriorly; the height of this tooth is 45 millims. The other specimen (B.M., m. 8593A), of which the anterior end is broken off, is a much older tooth, having all except the three last ridges in wear.

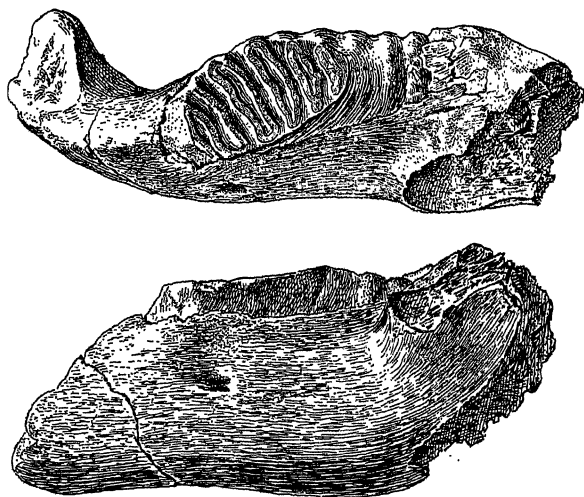
Last True Molar (m. 3).—Only two specimens were obtained of molars believed to be the ultimate ones of the lower series, on account of their superior size and greater number of ridges compared with the second true molars mentioned above. These are in a perfect state of preservation, both being of the left side and in much the same condition of wear. One (B.M., m. 8591, Plate 22, figs. 6 and 6A), an isolated tooth, is very narrow, the greatest width of the crown being 26 millims., while that of the other specimen (B.M., m. 8589) is 29 millims. Allowing for one ridge having been partially worn away anteriorly in both cases, the former consists of thirteen plates and the latter of twelve.

The ramus in which this last is situated (text-figs. 2 and 3) is broken off just behind

* 'Monograph on the British Fossil Elephants,' London, 1877-81, Part 2, pp. 134-139, figs. 4-32.

† 'Zool. Soc. Trans.,' vol. 9, Plate 5, fig. 2.

the molar, exposing a septum of bone overlying the posterior end of the tooth. This shows on its hinder surface a smooth, circular indentation which it was thought, at first sight, might have been caused by an advancing tooth, in which case the specimen in question would necessarily be the penultimate instead of ultimate molar. However, in view of its early stage of wear—there being six plates still untouched—it is improbable that this depression was so caused. The late development of the tooth destined to take the place of the one in use is well shown by several examples in the collection and it seems that frequently, if not invariably, almost the entire crown of a molar is in wear whilst its successor is as yet only partially formed. One of these



Figs. 2 and 3.—Crown and side views of left mandibular ramus containing a last true molar, B.M., m. 8589 (two-thirds nat. size).

specimens is that of an imperfect left mandibular ramus containing a somewhat damaged last milk molar, which very possibly had the total number of its ridges in wear (B.M., m. 8597). Behind this is a single germ plate of the succeeding tooth, its evident isolation and small size indicating that it had been in existence but a very short time. The right mandibular ramus (B.M., m. 8590, Plate 21, fig. 1) with a first true molar in position, to the rear of which is a large cavity enclosing a small germ tooth plate, has already been referred to (p. 354). A second true molar situated in another right mandibular ramus (B.M., m. 8588, Plate 21, figs. 3 and 3A) has the surfaces of all but the two last ridges abraded from use, nevertheless the following

ELEPHAS CYPRIOTES FROM A CAVE-DEPOSIT IN CYPRUS.

tooth is represented in the cavity behind by only two germ plates not yet cemented together.

Compared with those of *E. melitensis* the two above-mentioned last lower molars of *E. cypristes* are somewhat smaller, both in antero-posterior length and in the width of their crowns. Dr. LEITH ADAMS calculated the entire length of one of the former* to have been 5·4 inches and also describes and figures another 6 inches in length (= 151 millims.).† The same measurement in the Cypriote specimens cannot be taken absolutely correctly but they had approximately a total length of 125 millims. (in B.M., M. 8589) and 114 millims. (in B.M., M. 8591) respectively.

Limb Bones.—The only limb bone obtained that has been determined as that of *E. cypristes* is the distal extremity of a femur, which is unfortunately too imperfect to admit of a description being given.

Conclusions.

Although from an examination of the teeth alone conclusive evidence cannot perhaps be deduced as to the relationship of the Cypriote elephant to other forms, still several interesting and suggestive points may be noticed. The characters of the dentition of *E. cypristes* do not point to any connection with the recent Asiatic species, from which this pigmy form differs markedly in the lowness of its ridge formula and in the simple construction of the enamel of the molars.

Undoubtedly there is a strong resemblance between the teeth of *E. cypristes* and those of the Maltese and Sicilian forms, more especially *E. melitensis*, but this likeness is very apt to be over-estimated owing to the respective dwarf proportions of these island races. The Maltese pigmy species have been considered to be most closely allied to *E. antiquus* and *E. africanus*.‡ On the other hand it seems more probable that *E. cypristes*, which so far as available material is concerned shows no close affinity to the African species, is rather connected with *E. antiquus* and *E. meridionalis*, agreeing with this last in the lowness of its ridge formula,§ though differing in wanting the persistence of the strongly marked digitation of the plates which is usually found in the molars of that elephant.

In this connection it is interesting to note that from the characters of the carpal and tarsal bones (the only remains known) of *E. lamarmoræ*, the dwarf elephant of Sardinia, Dr. C. I. FORSYTH MAJOR considers it to have been most closely allied to *E. meridionalis*|| It is perhaps also noteworthy that the remains of *E. cypristes*, as well as those of *Hippopotamus minutus*, appear to vary but little in size, whereas in

* Figured in FALCONER, 'Pal. Mem.' vol. 2, Plate 12, figs. 4 and 4A.

† 'Zool. Soc. Trans.,' vol. 9, pp. 30 and 31, Plate 6, figs. 1 and 1A.

‡ See 'Cat. Foss. Mamm., Brit. Mus.' R. LYDEKKER, 1866, Part 4, p. 138.

§ The agreement in this respect with *E. meridionalis* has already been pointed out by Dr. LEITH ADAMS in the case of the Maltese dwarf species, 'Zool. Soc. Trans.,' vol. 9, p. 112.

|| "Die Tyrrhenis," 'Kosmos,' vol. 7, 1883, p. 7.

the case of the dwarf species of elephants and hippopotami from Malta and Sicily this occurs to a considerable extent, so much so in the case of the latter that molars may be found of intermediate sizes connecting *H. melitensis*,* *H. pentlandi* and *H. amphibia*.†

Since writing the first notice of *E. cypriotes* I have come to the conclusion that the average number of plates in the true molars is somewhat less than was at first supposed, therefore the corrected ridge-formula, exclusive of talons, would stand as follows: $\div$, $\frac{5}{3}$ $\frac{7-8}{7-8}$ $\frac{7-8}{7-8}$ $\frac{8-9}{8-9}$ $\frac{11-12}{11-12}$

This is slightly lower than that of *E. melitensis* which Dr. LEITH ADAMS, later than Dr. FALCONER and after examining a further large amount of material, gives as $\frac{3}{3}$ $\frac{5}{5}$ $\frac{8-9}{8-9}$ $\frac{8-9}{8-9}$ $\frac{10}{10}$ $\frac{12}{12}$

The marked lateral compression of the tusks of *E. cypriotes* (Plate 22, fig. 9), which is a constant character in all the specimens so far obtained, would in itself be almost sufficient to distinguish this from the other pigmy elephants of the Mediterranean region. Specimens of the penultimate and last milk molars (m.m., 3 and 4) are somewhat larger than those of *E. melitensis*, but from the size of the true molars of these species it is believed that the latter must have been slightly the larger animal of the two. Compared with examples from Malta in the collection of the British Museum, the isolated tooth plates of *E. cypriotes* appear to be smoother and more faintly sculptured both anteriorly and posteriorly and also less strongly digitated, thus causing the generally simpler pattern of the crowns of the worn molars.

Presupposing that the dwarf elephants of the Mediterranean region were all derived from a common ancestor, the simpler construction of the molars of *E. cypriotes* might be explained on the assumption that this species was isolated and subsequently differentiated from the parent stock, prior to a similar fate overtaking the Maltese and Sicilian races. This is borne out by the fact that *E. cypriotes* lived contemporaneously with *H. minutus*, a more generalised and primitive form‡ than either *H. pentlandi* or *H. melitensis*, the associates of the pigmy elephants of Malta and Sicily.

The geological evidence, as distinguished from the palæo-zoological, also supports this theory, for it appears probable that Cyprus became an island at an early period. On consulting the Admiralty Chart it will be found that there is no submerged bank indicating a recent connection with the neighbouring mainland, but that, on the contrary, the 200-fathom line is reached within a very short distance from the land round the whole of the coast line. This hypothesis is likewise strengthened by the

* Dr. FORSTER MAJOR, 'Geol. Mag.', (IV.), vol. 9, 1902, p. 198.

† 'Cat. Foss. Mamm., Brit. Mus.,' Part II., 1885, p. 292.

‡ See Dr. FORSTER MAJOR, 'Zool. Soc. Proc.', vol. 2, 1902, p. 107.

characteristics of the recent terrestrial fauna and the avifauna, both of which groups include a number of distinct races peculiar to the island. Further, as Dr. FORSYTH MAJOR* has already pointed out, KOBELT† was led to the same conclusion from his investigations of the recent mollusca of Cyprus. Of the bearing of the flora on this question I cannot speak, but I believe that here also are included a number of forms confined to this locality.

On the other hand Malta appears to have been, comparatively speaking, quite recently connected with Sicily, and consequently with Italy to the north, and southwards with the north of Africa. The actual submerged lands which formed the highways between these different points were discovered by Admirals SMYTH and SPRATT and a chart showing their position and present depth below the sea was included by the latter in a paper on the Maltese bone-caves.‡ This proof of the former continuity of land between Malta and Sicily may well account for the occurrence of the same species of elephant—*E. mnaidriensis*—and also of hippopotamus—*H. penlandi*—in the cavern deposits of both islands.

I should like to take this opportunity of expressing my sincere thanks to all those who have done so much to help me, both whilst in Cyprus and when working out some of the results of my excavations in the island. These are more especially due to Mrs. CLARENCE WODEHOUSE, with whom I went to Cyprus, to Dr. HENRY WOODWARD, F.R.S., to the members of the Government Grant Committee for enabling me to continue my explorations, and to Dr. ARTHUR SMITH WOODWARD, F.R.S., for so kindly giving me access to specimens in the collection of the British Museum; also to Dr. C. I. FORSYTH MAJOR and Dr. C. W. ANDREWS for valuable assistance and advice whilst working out my collections.

* 'Zool. Soc. Proc.,' vol. 2, 1902, p. 110.

† 'Studien sur Zoogeographie,' II, 'Die Fauna der Meridionalen Subregion,' pp. 337-339, 1898.

‡ 'Geol. Soc. Quart. Journ.,' vol. 23, 1867, p. 293.

EXPLANATION OF PLATES 21 AND 22.

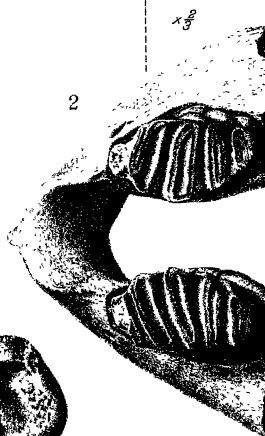
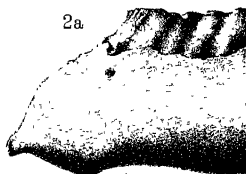
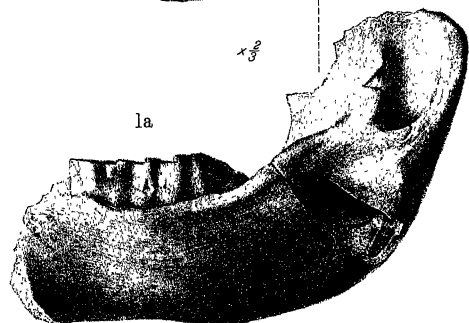
PLATE 21.

- Fig. 1. Crown view of first lower true molar in right mandibular ramus.
B.M., m. 8590, p. 354.
- Fig. 1A. Side view of ditto.
- Fig. 2. Crown view of portion of mandible with two first (?) true molars.
B.M., m. 8596, p. 354.
- Fig. 2A. Side view of ditto.
- Fig. 3. Crown view of second true molar in right mandibular ramus.
B.M., m. 8588, p. 355.
- Fig. 3A. Side view of ditto.
- Fig. 4. Crown view of upper first true molar. B.M., m. 8602, p. 351.
- Fig. 4A. Side view of ditto.
- Fig. 5. Crown view of penultimate lower milk molar. B.M., m. 8597, p. 352.
Natural size.
- Fig. 6. Section of portion of upper true molar. B.M., m. 8618, p. 353. Natural size.
- All figures are two-thirds natural size except Figs. 5 and 6 which are natural size.

PLATE 22.

- Fig. 1. Crown view of second upper true molar. B.M., m. 8599, p. 351.
- Fig. 1A. Side view of ditto.
- Fig. 2. Crown view of third upper true molar. B.M., m. 8600, p. 352.
- Fig. 2A. Side view of ditto.
- Fig. 3. Crown view of penultimate milk molar with portion of left maxilla, showing
alveolus of anterior milk molar. B.M., m. 8617, p. 350.
- Fig. 4. Crown view of last upper milk molar. B.M., m. 8608, p. 351.
- Fig. 4A. Side view of ditto.
- Fig. 5. Crown view of last milk molar in left mandibular ramus. B.M., m. 8595,
p. 353.
- Fig. 5A. Side view of ditto.
- Fig. 6. Crown view of last lower true molar. B.M., m. 8591, p. 355.
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- Fig. 7. Immature incisor ♀. B.M., m. 8608, p. 350.
- Fig. 8. Incisor ♂. B.M., m. 8614, p. 349.
- Fig. 9. Section of incisor. One-third natural size.

All figures two-thirds natural size except Fig. 9 which is one-third natural size.





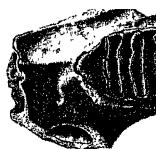
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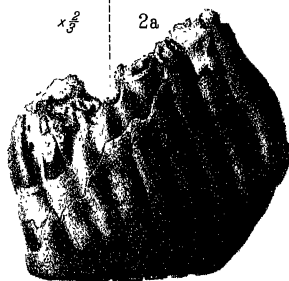
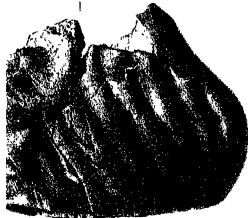


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5

x 8



x 8



5a



7

x 8



8

x 8

9

INDEX, SLIP.

ELLISOT, (Capt.) R. H.—A Contribution to the Study of the Action of
Indian Cobra Venom. Phil. Trans., B, vol. 197, 1906, pp. 361-406.

Cobra Venom—Action on Blood-vessels, Heart, Respiration, and Cardio-
respiratory nervous supply.

ELLISOT, (Capt.) R. H.
Phil. Trans., B, vol. 197, 1906, pp. 361-406.

XIII. *A Contribution to The Study of the Action of Indian Cobra Venom.*

By Captain R. H. ELLIOT, *M.B., B.S. Lond., F.R.C.S. Eng., D.P.H. Camb., etc., of the Indian Medical Service, on Special Duty for Snake Venom Research under the orders of the Rt. Hon. The Secretary of State for India.*

Communicated by Sir THOMAS R. FRASER, M.D., LL.D., F.R.S.

(From the Materia Medica Laboratory of the University of Edinburgh.)

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At the suggestion of Sir THOMAS FRASER, H. M. Secretary of State for India very kindly placed me on special duty for Snake Venom research in the Pharmacological Laboratory of the University of Edinburgh. Professor FRASER, under whose superintendence I was directed to work, suggested that I should carefully study the pharmacology of Indian Cobra venom, and the work herein set forth is the outcome of that suggestion. Professor SCHÄFER also very kindly placed his laboratory at my disposal, and both to him and to Sir THOMAS FRASER I owe my most cordial acknowledgments for their unvarying kindness and for their very valuable and always ready assistance.

Previous Work on the Subject.

Some of the earliest work done on the pharmacological action of Cobra venom was that by LAUDER BRUNTON and FAYRER,* published about thirty years ago. They considered that Cobra venom selected the cerebro-spinal nerve centres for its seat of action; it paralysed these, and in large doses it acted also on the ganglia of the heart, causing arrest of cardiac action. They laid little stress on the rôle played by circulatory failure, and in support of their views they quoted a number of experiments in which cardiac pulsations continued after the apparent death of the animal. In volume 22 they go on to discuss the subject still further. With the facts at their disposal they were unable to come to any definite conclusion as to the exact influence of the venom on the heart, but they thought that the heart's arrest in systole, which they at times observed was due to "some action on the cardiac ganglia." They drew attention to the great difference observed in the effect of venom according as it was applied to the surface or to the interior of the heart. In this connection they quote an experiment in which they perfused the frog-heart with a solution of Cobra venom, and obtained arrest of it in a position midway between systole and diastole. The strength of solution used is not stated and details are wanting, but the experiment is of great interest, as it is the only record I can find in any writer's works of an attempt to perfuse an isolated part with Cobra venom. They thought the action was due to an influence on the cardiac ganglia. They found that in large doses Cobra venom destroyed the inhibitory power of the vagus, which it did not do in small doses. That the inhibitory branches of the vagus were sometimes affected they were confident, but they do not seem to have been able to differentiate clearly between a direct action of the venom on the vagal system, and the indirect effects brought about by means of interference with respiration. In dealing with the last-named subject, they clearly observed the paralysis of the phrenic nerve ends, and divided the responsibility for respiratory failure between this and a direct interference with the centre in the medulla oblongata.

It is difficult to do justice within one's limits of space to these papers, which will ever remain classics in Snake-venom literature, and only two more points can be touched-upon. The first is the conclusion, founded on only two experiments, that the rise of blood pressure in cobraism was due to contraction of the arterioles opposing a barrier to the exit of blood.

The second point referred to is the influence of artificial respiration in restoring function to a cobraised animal. Lastly I would venture to offer, with the greatest respect, a few criticisms on this work, for it was those very criticisms which led me to go over again some of the ground BRUNTON and FAYRER had covered. Firstly they do not seem to have standardised the venoms they worked with; secondly they omitted to accurately graduate their dose to the weight of animal used, and thirdly

* 'Roy. Soc. Proc.' vols. 21, 22, and 23.

they did this work about thirty years ago, when physiological and pharmacological knowledge had not reached the position it enjoys to-day, and when some of our present methods of experiment were unknown or nearly so. CUNNINGHAM, in "Scientific Memoirs by Medical Officers of the Army of India,"* advanced the view that Cobra venom acted on the respiratory mechanism through the blood, and not through the nervous system. He has found few supporters.

The next important paper on the subject is that by WEIR MITCHELL and REICHERT, published in the 'Smithsonian Contributions to Knowledge,' of 1890. Like BRUNTON and FAYRER they appear to have omitted to standardise the specimens of venom they used, or to calculate the dose to the body-weight. Farther, the doses of venom used were far in excess of those a snake-bitten man would receive, for death ensued in less than 20 minutes in a large number of the experiments, and life seldom far exceeded this period of time after the injection. The same objection applies to a large number of the experiments which other observers have made whilst working at this subject; and yet it is obvious that the dosage in which a poison is usually received is a very important factor in the determination of the effects produced. It is unsafe to argue as to the action of any poison from a consideration of the symptoms, etc., produced by it in a concentrated form.

WEIR MITCHELL and REICHERT observed a temporary fall of blood pressure, followed by a decided rise thereof, which eventually yielded to a final fall. The first fall they attributed chiefly to "depression of the vaso-motor centres," but they thought it may be partly cardiac. The rise they considered "capillary," and the final fall cardiac.

They believed that two antagonistic factors are at work on the rate of the heart, viz., an increased activity of the accelerator centres tending to quicken the beat, and a direct action on the heart tending to slow it. The former influence is apparently called in to explain those cases in which they gave doses fatal in very short periods of time and found marked quickening of the beat.

They ascribed death in most cases to paralysis of the respiratory centres and seem to attach no weight to an affection of the phrenic nerve-ends. They laid stress on the influence of the venom on the vagal peripheries whereby respiratory effort is increased. It is only fair to add that their paper does not primarily concern itself with Cobra poison, and that it is a difficult, if not impossible, task to deal with many varieties of venom at one and the same time. Recent research has clearly shown that this is so.

The last important paper which calls for review is that by RAGOTZI.† RAGOTZI shows that in order to produce peripheral paralysis it is necessary to carefully graduate the amount of venom used, avoiding a rapidly fatal dose on the one hand and one which is too weak on the other.

* 1895, Part IX., and 1898, Part XI.

† Virchow's 'Archiv,' vol. 122, p. 201.

He found that artificial respiration might keep warm-blooded animals temporarily alive, but that it would not lead to recovery. In such animals the endings of the phrenics and of the trunk-muscle nerves were paralysed, the paralysis being of such a nature that prolonged artificial respiration did not bring about recovery of the nerve-ends.

He does not think that the failure of respiration is due to an affection by the venom of the respiratory centre, but holds the motor nerve-ends alone responsible. In marked contrast to Curara, he finds that Cobra venom spares the vagal centres and nerve-ends.

In his blood pressure work he used very heavy doses of venom, if one may judge from the rapidity of cessation of respiration; the dose per kilo. is unfortunately not stated. He observed that artificial respiration affected the blood pressure, and he therefore concluded that the vaso-motor centre had remained active.

He observed that large doses of Cobra venom stopped the frog-heart in systole. In the mammal strong doses of venom lessened the amount of the heart's movements, whilst smaller doses increased it. To kill the heart in diastole small doses, subcutaneously given, were required. He surmised that systolic death must be due to a direct action of the poison on the heart-muscle, and that "diastolic death" is to be attributed to paralysis of the intracardiac ganglia. He did not find the vagal ends in the heart affected.

C. J. MARTIN's able article in ALLBUTT's 'System of Medicine,' is also of considerable interest, although the writer concerns himself largely with other than Indian snakes. Working with an open thorax, he finds that after the injection of Viperine venom the heart beat is first increased and later diminished. If the heart is cut off from all centric influences, by cutting the cardiac nerves, no primary increase occurs. In Cobra poisoning alone he believes that the circulatory mechanism is not easily affected, the heart beating strongly even after respiratory death. All venoms produce a sudden fall of blood pressure if in large doses. In *Pseudechis* poisoning, the cause of the primary fall, as shown by simultaneous tracings of carotid blood pressure and of the volumes of the spleen and kidney, is mainly cardiac. If vascular dilatation of the abdominal area occurs at all, it is masked by cardiac depression. He states that an excised frog-heart fed with dilute solutions of venom stops in diastole, while if the venom solution is more concentrated, it at once stops the heart in partial systole. The kind of venom is not stated, but from the context I infer that it was Viperine.

MARTIN finds that vagus stimulation stops the heart in Cobra and *Pseudechis* poisoning up till near the end of life. In his views on the action of Cobra venom on the respiratory system, MARTIN closely follows WEIR MITCHELL and REICHERT.

I greatly regret that exigencies of space prevent me from discussing at much greater length the previous valuable work on this subject which has been done by others.

Object of this Research.

The main object of this research was to try to fill in some of the gaps still left in our knowledge of the pharmacology of Cobra venom. I particularly desired to ascertain the precise part played by the various important centres, nerves and organs, in the production of death from cobraism. To this end I made it my first object to study the action of the venom on the isolated frog-heart and on the vessels of frogs whose central nervous system had been destroyed. The same structures were then experimented on in frogs whose brains above the medulla oblongata had been removed. The isolated mammalian heart was next submitted to the action of the venom, and finally, the ground having been thus cleared, more complicated experiments were undertaken on various living mammals.

The minimum lethal dose per kilo. of body-weight of the specimens of venom used was carefully calculated at an early stage of the research, and to save repetition and explanation, the doses quoted throughout this paper are stated in fractions or multiples of the M.L.D. for the particular animal under discussion.

Perfusion of the Vessels of the Frog.

The animal was always carefully and thoroughly pithed at least half-an-hour before commencing the experiment. A continuous record was taken of the amount in cub. centims. of the escaping fluid. In the earlier experiments a solution of sodium chloride in Edinburgh tap water (·6 per cent.) was used for the normal, and also as a vehicle for the venom. It was found that with this solution slight but steady dilatation of the vessels took place after long perfusion, and RINGER's fluid, as modified by RUSCH, was therefore substituted for it.* The result was satisfactory.

A 1/50,000 solution of Cobra venom reduced the flow in a little over an hour from 1·3 cub. centims. per minute to ·3 cub. centim. in the same time. The following solutions were then tried in turn :—1/100,000, 1/300,000, 1/1,000,000, 1/2,500,000, 1/5,000,000, and 1/10,000,000. A constriction of the vessels was distinctly observed even down to the last and weakest strength, in which indeed the flow was halved in less than an hour. Solutions of the venom at 1/15,000,000 and at 1/20,000,000 gave negative results.

The next step is the estimation of the concentration of Cobra venom in the blood of a bitten man. This can only be made approximately. CALMETTE has calculated that the larger venomous snakes, such as the Cobra, yield on an average about ·050 gramme of venom (weighed in the dry) at each bite. He thinks this a liberal estimate, and he infers that the fatal dose for a man is about ·010 gramme.†

* *Vide* Pfüger's 'Archiv,' vol. 73, p. 546.

† *Vide* Calmette's note to the article on Venom in Allbutt's 'System of Medicine.'

Sir T. FRASER has calculated an average of '255 grammes' of Cobra venom for each bite, but thinks an average of '195 grammes to be probably a truer estimate. Reckoning on the cat-basis, he places the M.L.D. for man at '317 grammes, but again he thinks a lower estimate more correct, and gives '0317 grammes as nearer the true mark. The duration of life after Cobra bite in most of the fatal cases leads one to think that but little over the minimum lethal dose is usually injected. The margin between CALMETTE's average amount for a bite and FRASER's minimum lethal dose is by no means large, when the difficulties of the investigation are considered. We shall probably err on the side of safety if we consider that a fatal dose for an average man is about 30 milligrammes, as this figure is covered by both the estimates given above. Assuming the weight of an average man to be about 70 kilos., and the weight of his blood to be about one-thirteenth of this figure or rather less than 5.5 kilos., and assuming also that the whole dose of venom injected is absorbed into the circulation, we find that the concentration of the poison solution in the body will be about 1 : 183,333.

Even if the above calculations are considered to be over-liberal, or if it is objected that the whole quantity of venom would not be in circulation at one time, we have still a very wide margin between 1/183,333, the figure we have just arrived at, and 1 : 10,000,000, which has been found to be a sufficiently strong solution to act on the isolated vessels within an hour. Two additional facts are to be borne in mind, viz., that the tissues of a victim of snake bite are exposed to the action of the venom for many hours, and that it is extremely difficult to dislodge Cobra poison from those tissues, once it has fastened on to them. In illustration of the last point, it may be mentioned that isolated vessels or isolated hearts, which have been submitted to long perfusion with the venom, cannot be recovered by perfusion with a normal fluid; farther, if the original venom perfusion has been short, and if recovery under normal fluid takes place, a second irrigation with the lethal fluid acts much more quickly and decisively than did the first one.

It is clear, therefore, that the sustained high pressure, which is so marked a feature of blood-pressure tracings in slow death from cobraism, is explainable without going any farther than the direct action of the poison on the walls of the vessels. The present state of physiological knowledge does not permit of a discussion of the exact method of this local action. To my volunteer assistant, Mr. JOLLY, my acknowledgments are due for his valuable help in the work of this section of my paper.

Perfusion of the Frog-heart with Cobra Venom.

In order to test the action of Cobra poison on cardiac tissue, SCHÄFER's plethysmograph was used for frog-hearts. The instrument was modified by substituting a metal for a glass cylinder for the piston. Moreover, oil was done away with in the body of the instrument, and Ringer's fluid used in its stead. The fluid used for

perfusion consisted of one part of defibrinated ox-blood and two parts of RINGER's fluid (RUSCH's formula already quoted). This was used for the normal and also for conveying the venom, both portions of the solution being taken from the same bottle each time.

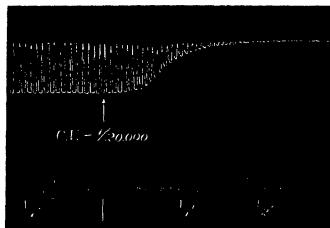
There is nothing of note to be said about the method of removing the heart from the body and fastening it into the plethysmograph, except that air was most carefully excluded from the interior of the organ.

In dealing with strong solutions of Cobra venom, and by this term must be understood anything stronger than 1/100,000, the duration of a perfusion experiment is to be reckoned in seconds or, at the outside, in minutes. The beat of the heart is quickened, and the organ passes into a condition of systolic tone in which it speedily dies. The diastolic end of each excursus becomes progressively shortened, whilst the systolic end remains the same or may even be lengthened. It is not possible within the limits of this paper to attempt even a summary of the mass of tracings which have been taken, but a few typical experiments have been selected for analysis, the original records being preserved in the Archives of the Royal Society (Plate I.).*

On 29.10.03 a heart was perfused with blood mixture till a normal was obtained, and a 1/10,000 solution of Cobra venom in the same medium was then run through. The normal shows four beats in 20 secs. 1 min. after venom was turned on, the beats had risen to seven in 20 secs. ; 1 min. later the figure was 9.5 for the same period; and after 3 mins. of venom perfusion the organ was dead in tight systolic position.

On 31.10.03 a heart was similarly perfused with a solution of 1/50,000 of the venom. Before venom was run in there were four beats in 20 secs. ; 2 mins. after poison was admitted there were 5 beats in 20 secs. ; 4 mins. after poison was

Extract 1.



Extract from a Tracing of a Perfusion of a Frog-heart with a solution of Cobra venom 1/20,000. Systole is upwards. The arrow shows where the venom was run in. Time is marked in seconds and minutes.

admitted there were 6.5 beats in 20 secs. ; 5 mins. after poison was admitted the heart was in tight systolic position.

* These references to plates are to the originals, from which only extracts are reproduced in this paper.

On 2.11.03 a solution of 1/100,000 was used for perfusion. Within 7 mins. of the admission of the poison, the rate of heart-beat had more than doubled, and in 20 mins. the organ was dead in tight systolic position.

In all of the above experiments an early contraction of the excursus took place, and in each case it will be observed that the shortening was at the expense of the diastolic portion of the curve, and never at that of the systolic portion. In the first experiment the contraction had begun in 30 secs., in the second in 100 secs., and in the third in less than 3 mins.

TABLE designed to show the Influence of comparatively weak Solutions of Cobra venom in increasing the Rate of Beat of the isolated Frog-ventricle, when perfused through its Cavity.

Time in minutes before venom-perfusion commenced.	10 9 8 7 6 5 4 3 2 1	9 9 9 9 9	9.5 9 8 8.5 8.5	7.5 7.5 7.5 7.5	5.75 5.75 6 5.75 6 6.5 6.5 — 7 6.25	9 8.5 8 8.3 8.5 8 — 8 8 8.5	10 10 8.5 8 7.5 7.5 6.5 6.5 6.5
1/500,000*							
Time in minutes after venom- perfusion had commenced.	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	9.25 11 13 14 14 14 14 9 9 9.5 9.5 9 10	8.5 — 10 9.5 10.5 9.5 8.5 9 9 9.5 9.5 9 10	8 10 10.25 10.25 10.25 10.5 10 9 9 9 8.5	6.25 7.25 8.75 9 9.25 9 9 9 9.5 10 10 10 10 10	8 8.75 9 9 10 9.5 9 9 9.5 10 10 10 10 10	6.75 7.5 7.75 8.0 8.5 9 9 9.5 9.75 10 — — — 11

* Strengths of solutions of cobra venom used for perfusion.

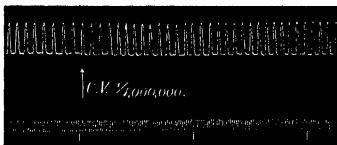
The figures in the first column give the number of minutes before and after the perfusion of venom solution. The remaining columns (one for each experiment) give the number of beats per minute of the isolated frog-ventricle in successive minutes. The strengths of the different solutions of venom used are also shown.

We pass next to the consideration of the effects of Cobra venom in more dilute solutions, and are now confronted with new difficulties. A heart which is perfused for 2, 3, 4, or more hours shows definite changes in its tracing, even to a superficial observer. When one comes to closely analyse such a record, and

to count the beats in successive minutes throughout, one finds that the rate of beat varies more than one had suspected. There is in fact a gradual tendency for the heart to beat faster at first and slower later on. At the same time its excursions slowly and steadily diminishes, and the organ drifts towards a position of diastole. Rarely one finds a heart pass very slowly towards the systolic position, but this is almost certainly due to some change in the blood used. Such a sample of nutrient fluid should, of course, be rejected, as it introduces fallacies into the research. Bearing the above facts in mind, one had to make a large number of tracings, both normal and otherwise, and to carefully exclude all those into which fallacies, due to the changes above described, had apparently crept.

Some of these results are given in the subjoined table, and were selected from tracings in which the heart was either very steady, or actually slowing, at the time venom was introduced. (For tracings illustrating this section see Plate II.) Briefly, the case may be thus stated. Solutions of Cobra venom of a strength of $1/250,000$, of $1/500,000$, and even at times of $1/1,000,000$ increase the rate of heart beat and kill the organ in a position of systole. With the last-named strength this result is, however, far from a certainty, but the influence of the venom in "toning up" and quickening a heart, especially if feeble before, is unmistakeable. Solutions of a

Extract 2.



Extract from a Frog-heart Perfusion Tracing, with a $1/1,000,000$ solution of Cobra venom, at the time of running in the venom. Time marked in seconds and minutes.

Extract 3.



From the same experiment as Extract 2, but 25 minutes later.

strength of $1/5,000,000$ and of $1/10,000,000$ show no tendency towards producing systolic tone, but they quicken the rate of heart-beat, and make it very regular. Even a weaker solution ($1/50,000,000$) has been shown to have the same action, but the experiment requires repetition.

For comparative purposes, experiments were next made with a very pure specimen of strophanthin, kindly given me by Sir T. FRASER. This was selected as being the most powerful known glucoside of the group to which it belongs. It has also the virtue of constancy in action. The tracings on Plate III. speak for themselves. They show that the action of the glucoside is much slower than that of the venom. (Compare plates I. and II. with III.). The tendency to diastolic phases alternating with periods of systolic tone are also noteworthy. The main features of similarity of

action of these two powerful poisons are, however, sufficiently striking, and closely ally them in their essential properties.

As an allied substance, acokantherin (one of the famous arrow-head poisons) was also tested. It taught but little of interest for the purposes of this paper, and its action appears to be linked up to that of Cobra poison through the intermediation of strophanthin. The specimen of acokantherin was kindly given me by Sir T. FRASER.

Lastly, I studied the action of sulphate of atropia on the isolated heart. This drug has been recommended for the treatment of cases of snake-bite in India.

At first sight one would expect atropine and its salts to be of value by virtue of its action on the vagus nerve-ends in the heart; for blood-pressure tracings show clearly that the heart is slowed in cobraism by means of stimuli which reach it through the vagi. Practically, it was found that, whilst sulphate of atropia had a slight beneficial action on the heart in the early stages of a Cobra poison blood-pressure tracing, the opposite was the case later on in the experiment. The heart, under the influence of the drug, would suddenly collapse, and pressure would fall to zero with a run. Indeed, Dr. PRENTICE (Vans Dunlop Scholar of Edinburgh University) and I found that even the careful administration of the drug at such a stage was most hazardous. I had previously tested atropine in various doses on rats, rabbits, and frogs, which had received very low lethal doses of Cobra poison. The results were entirely negative, so far as any remedial action was concerned. All this is readily explained in the light of my recent work; I find that, whereas the sulphate of atropia in strong solutions (1/1000) causes death of the heart in diastolic position (probably a nerve-end action), the opposite is the case with weaker strengths. Thus a 1/5000 or a 1/10,000 solution quickens the heart and causes it to pass into a condition of systolic tone, in which the excursions is greatly diminished at the expense of the diastolic portion of the curve. This systolic effect is lost with still weaker solutions, or at least cannot be demonstrated in frog-heart perfusions.

If we now combine Cobra venom and sulphate of atropia in the same solution, taking care that each substance taken separately is well below the strength which is capable of throwing the heart into systolic tone, we find that the joint action of the two is that of a powerful systolic poison. Indeed, the result appears out of proportion to what might have been expected from a knowledge of each substance's separate action. In order to illustrate this point, the three tracings shown on Plate IV. were taken on the same day from the same specimens of blood and under the same conditions in all respects. The heart on which the atropine test was made was not a very active one.

The absence of any tendency towards systolic tone in this tracing and in that of the venom alone is conspicuous, whilst in the case where the substances are combined, it is very marked indeed. One admits freely that such a strength of atropine as was used here would never be tolerated *in vivo*, but on the other hand a much stronger

solution of venom might easily be present, and farther the action would be much more greatly prolonged. The main point is that the two substances may reinforce each other's action, and may thus possibly embarrass a heart. The sulphate of atropine would therefore appear to be a hazardous drug to use in cobra poisoned individuals.

I would take this opportunity of acknowledging the valuable help given me in my heart-perfusion work by my volunteer assistant, Mr. E. BURNETT, B.A. Lond.

Comparison of Cobra Venom with certain other Powerful Poisons.

Having carefully standardised the action of Cobra venom on frog-hearts and frog-vessels, in the manner already shown, one is in a position to compare this poison with other drugs which it resembles pharmacologically.

Professor T. R. FRASER* found that strophanthin exerted an appreciable action on the frog-heart in solutions as dilute as 1/20,000,000. He computed that such an action was 8 times as powerful as that of adonidin, scillotoxin, or erythrophleine; 20 times as powerful as that of helleborein, 30 times as powerful as that of convallamarin; 300 times as powerful as that of digitalin; 6000 times as powerful as that of saponin; and 30,000 times as powerful as that of sparteine and caffeine. He concludes his comparison with the following words:—"The action of strophanthin upon the heart is, therefore, more powerful than that of any other substance, with regard to which data exist wherewith to institute a comparison."

Dealing with the action of strophanthin on the muscular tissue of the blood-vessels, he compares this drug with digitalin. In solutions of the latter as dilute as 1/100,000, he finds an early and powerful constrictive action manifested, whilst strophanthin does not give a distinct action in solutions weaker than 1/20,000. I cannot find that he carried the testing of digitalin down to weaker solutions.

It is pertinent to remember that in dealing with Cobra venom, we are in all probability not handling the isolated poison. This makes it still more astonishing to find its action carried down to such extreme dilutions as 1/10,000,000 or less. Even as it stands, the venom will rank superior in rapidity, and probably fully equal in strength, to strophanthin in its direct action on the heart; whilst in its action on the vessels it is probably not inferior to digitalin.

Reading through Professor FRASER's paper, another point of considerable interest strikes one. It seems probable that the diastolic action he speaks of as found in strophanthin solutions may be due to the drug acting directly or indirectly on the vagal cardio-inhibitory mechanism, thus allying it still more closely with Cobra venom. This side of the action of Cobra venom will be dealt with later on in this paper.

* 'Roy. Soc. Edin. Trans.,' vol. 36, pp. 403 *et seq.*

The Action of Cobra Venom on the Frog-Heart in Situ.

Having ascertained the action of the venom on the isolated frog-heart, the next stage in the investigation was the study of the organ *in situ*. *Rana esculenta* was used throughout.

After the destruction of the central nervous system, the body cavity was filled with strong venom solution (.001 gramme = 1 c.c.), and the heart movements were recorded by the suspension method. No change in rate was observed. When the same solution was dropped continuously on the suspended heart for 10 mins. at a time, the organ passed into a state of moderate systole, and its rate slightly increased. The action is interesting, as it is in opposition to the usual tendency of a suspension tracing, which is towards diastole and slowing (Plate V.).

The next step was to inject Cobra venom subcutaneously into frogs, and to closely observe the changes in rate of the heart-beat. Contrary to what holds in mammals, one found that the heart was not slowed by the venom. On the contrary, a distinct increase in rate was first observed at an intermediate stage, whilst late in cobraism the organ was beating with a frequency but little below that of the normal. It was thought possible that a slowing, due to the descent of vagal impulses, might be masked in these experiments by another factor, such as the influence of the poison on the heart muscle. In order to settle this point, a very careful count was kept of the exposed and undisturbed heart in various stages of cobraism, both before and after the application of atropine. No quickening of the heart was in any case observed, but, on the contrary, a distinct slowing was found in most cases. This latter phenomenon is very difficult to explain. At all events, it was clear that no increase of cardiac inhibition was actively present, and that the only observable action of the venom was to quicken the heart by directly acting on its muscular tissue.

Some farther series of experiments were undertaken, in order to ascertain whether Cobra venom had any direct influence on the centres, trunks, or nerve-endings of the vagi. Frogs were injected subcutaneously with doses of Cobra venom, which would be lethal, (1) in less than 12 hrs., and (2) in the course of several days. The animals were killed at various periods, by destruction of the brain in front of the medulla oblongata, and the activity of the vagal centres, and of the vagal nerves, was then carefully tested in each case by means of a secondary current. The centres were tested by laying bare the medulla oblongata, and directly applying the electrodes to its surface. The minimal stimulus required to definitely stop the heart was noted carefully, the current being applied for 10 secs. each time. The vagi were exposed in the usual way, and raised on the electrodes with a minimum of disturbance. They were not dissected free from the two nerves which accompany them, as this would have involved unnecessary handling. The results of these experiments went to prove most clearly that neither the vagal centres, the nerves, nor the nerve ends were at all affected by venom. This result was obtained whether the poison was in sufficient

dose to kill within a few hours, or in 2 or 3 days. Nor could it be found that the stage of Cobra intoxication the animal had reached had at all influenced the question. Normals were, of course, freely taken from uninjected frogs on the same days, and under precisely the same conditions.

Lastly, Cobra venom was directly applied to the exposed medulla oblongata of frogs, whose brains above this region had been previously destroyed. The earlier applications were made in solutions of various strengths. As no effect was thus produced, the poison was applied in as concentrated a form as possible, only enough RINGER's fluid being added to dissolve the dry venom. This viscid fluid was then dropped on to the floor of the medulla oblongata. Muscular spasms at once occurred, but the suspension tracing showed that no slowing of the heart had taken place. It was evident that, under the conditions of the experiments, the vagal inhibitory mechanism of the frogs used (*Rana esculenta*) was unaffected by the venom.

One must bear in mind the frog's independence of its pulmonary respiration, especially in winter time. A cobraised frog will live on for hours, or even days, at any time of the year, after all perceptible signs of pulmonary respiration have ceased. Obviously, we have here a marked difference from the condition of affairs in the mammal, whose important medullary centres are speedily affected by any blood change brought about by deficient aëration. Moreover, these experiments were carried out during an Edinburgh winter, in a room whose temperature varied from a maximum of 56° F. to a minimum of some degrees below freezing point. For a large part of the 24 hours it was below 41° F.

In the summer I had performed a few experiments to study the action of Cobra venom subcutaneously injected into frogs, and I then found a distinct, though not very great, decrease in the rate of frog-heart beat under cobraism. In winter, as has been seen, I obtained no such decrease of rate. I am, therefore, prevented from drawing any definite conclusions from my winter work on the vago-inhibitory mechanism of the frog. Dr. PRENTICE, who was working last summer on similar lines, tells me that he, too, found some decrease of heart rate as the result of hypodermic injections of African Cobra venom.

Perfusion of the Mammalian Heart.

The number of experiments of this kind so far performed has been limited, but some very clear indications of the action of Cobra venom have been obtained. By the kindness of Professor SHERRINGTON and Miss SOWTON I was enabled to do a few experiments with their apparatus in Liverpool. This apparatus is fully described by them in the Report of the Chloroform Committee, which is printed as a supplement to the 'British Medical Journal' of July 18, 1903. The solution used for perfusion was as follows:—NaCl .9 per cent., CaCl₂ .24 per cent., KCl .42 per cent., NaHCO₃ .01 per cent., glucose, .1 per cent. The required quantity of Cobra

venom was dissolved in a portion of this fluid taken from the common stock. Care was taken to keep both the normal and the venom solutions at the same temperature, and under the same pressure. Cats and rabbits were used, and the isolated hearts were fed by tying the cut aorta on to the cannula, and perfusing through the coronary vessels; the aortic valves, of course, closed under the pressure.

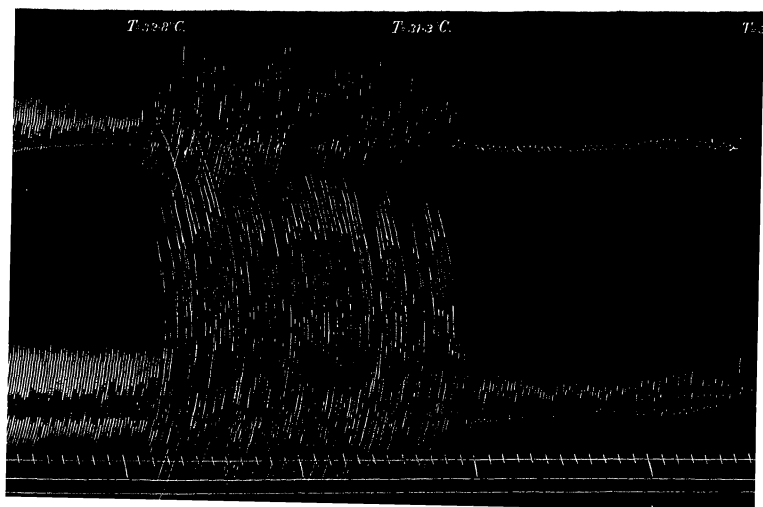
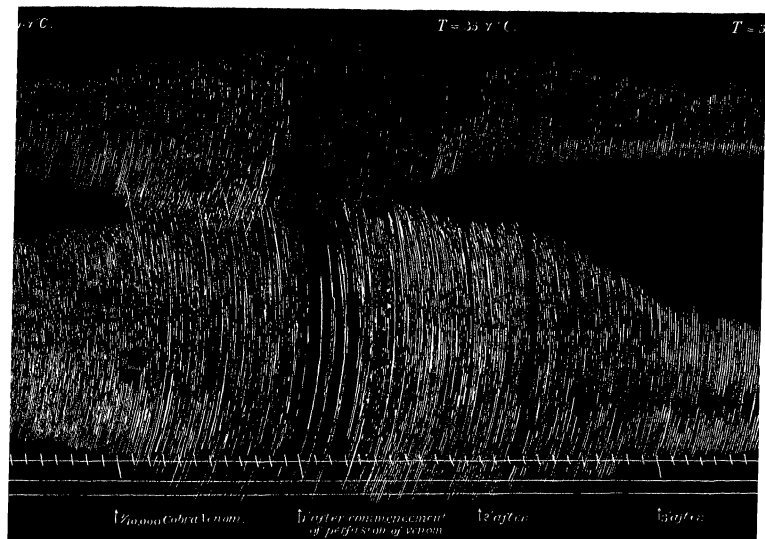
The following notes give a brief summary of some of the results obtained, and the original tracings are also furnished.

(1) A cat's heart was perfused with a solution of Cobra venom, 1/10,000. In less than 5 secs. both auricle and ventricle passed into a stage of violent excitement, which was so marked that the levers could not be kept on the drum; they over-rode its edges. This lasted 175 secs. The heart then steadied down, and beat with great regularity for 140 secs. A second period of excitement then ensued, and lasted 105 secs. The excursions of the beats rapidly diminished after this, and the heart had ceased beating 125 secs. later. After 85 secs., perfusion with normal fluid was resumed, and at the end of another 200 secs., the ventricle recommenced to beat, the auricle having preceded it by nearly a minute. The singular phenomenon was then observed, of the occurrence of 2 ventricular beats for each beat of the auricles. This did not last long, but is of interest, as it is sometimes observed during the course of a blood-pressure tracing taken on a cobraised animal. The onward passage of a wave of contraction is apparently obstructed in its course from the auricle to the ventricle in such a way that it enters the ventricle at two spots, an interval occurring between the two invasions. The result is that two waves of contraction enter the ventricle at each auricular beat. After the heart had been beating for 7 mins., and had again settled down to fair regularity, the venom solution was re-introduced, with the result that the excursions of both auricle and ventricle rapidly diminished. The latter had ceased beating in $1\frac{1}{2}$ mins., and the former in 4 mins., thus closing the experiment.

The tendency of both chambers to pass into a systolic phase was obvious, though masked, in both stages of the experiment. The flow through the coronary vessels decreased rapidly, and had almost ceased at the end of the experiment. This is, doubtless, to be attributed partly to the state of systolic tone into which the ventricle was thrown, and partly to a direct constrictive action of the venom on the coronary vessels. Any delay in perfusion will, obviously, lead to a cooling of the heart, and it has been abundantly proved that cooling at once slows the organ. The result is, that the tendency of the venom to increase the rate of the heart is in all these experiments largely masked. Careful analysis of a number of tracings shows, however, that the tendency to quickening of the beat is actually present. This brings the action of Cobra venom on the mammalian heart into line with what we have seen occurs in the frog-heart.

(2) A cat's heart was perfused with a solution of Cobra venom of a strength of 1/50,000. The amplitude of the ventricular excursions began to increase almost at once, reaching its maximum between the 11th and 20th mins. It then decreased

Extract 4.



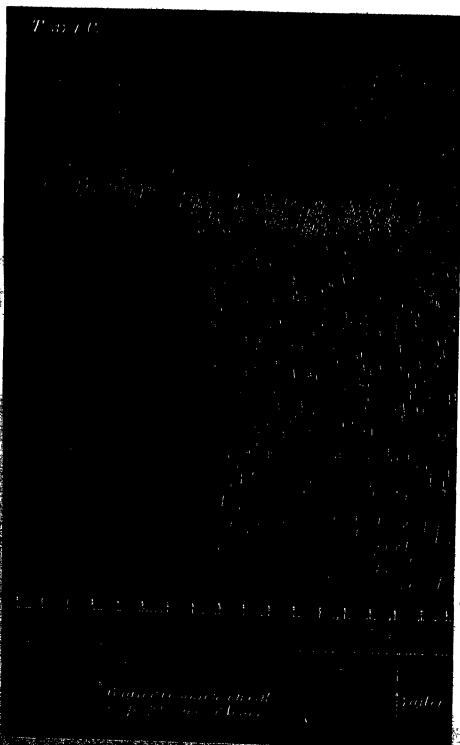
rapidly and the beat had practically ceased 7 mins. later. The auricle was much slower in first becoming affected, and also continued beating about 1 min. after the ventricle had ceased. Phases of excitement with intermissions are observable throughout both the auricular and ventricular tracings, but are much better marked in the latter than in the former. Both chambers showed a decided tendency towards systolic tone, as may be seen on the record. The same phenomena, dependent on constriction of the coronary circulation, again appear on this tracing.

(3) A cat's heart was perfused with a solution of Cobra venom of a strength of 1/100,000. The heart showed a tendency to beat in groups whilst being perfused

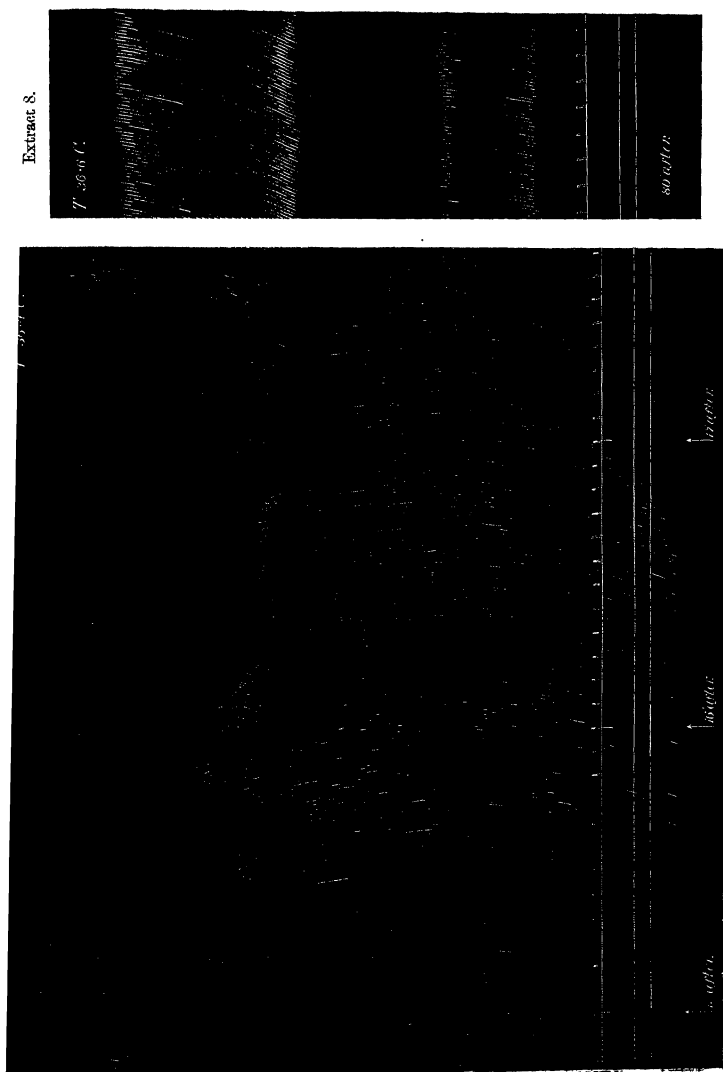
Extract 5.



Extract 6.



Extract 8.



with the normal fluid. One minute after venom solution was admitted, the auricular excursus (which alone was recorded), began to show signs of excited cardiac action. This stage lasted 7 mins., and was succeeded by an intermediate phase in which the heart became much more regular than it had hitherto been. This lasted another 7 mins. The heart then became more irregular than ever, and ceased to beat about 9 mins. later. The tendency of the organ to pass into systole was well marked.

(4) A cat's heart was perfused with a solution of Cobra venom of a strength of 1/500,000. Within 2 mins. of the venom reaching the heart, both beats (auricular and ventricular) commenced to increase in volume, and displayed a decided rhythm in so doing. Four and a half mins. later the ventricle became suddenly and violently excited. This stage lasted 11 mins. and an increase in the rhythmical variation of the strokes was observed. At the same time the auricle was passing through a similar but less pronounced phase of excitement. It is of interest to notice that the periods of rhythmical excitation and remission are not synchronous in the records of the two chambers. The heart at first showed quickening of its beat, but this was soon masked by the cooling consequent on systolic retraction. For over an hour a steady, even and regular beat was maintained, but the excursus gradually diminished. The experiment was stopped by the venom solution running out. When allowance is made for the interference with the coronary circulation, this tracing reminds one strongly of the results yielded by frog-hearts, for the organ after a preliminary period of excitement is first steadied and then slowly poisoned. It is important to remember that a strength of 1/500,000 of venom might possibly be present in the circulation of a Cobra-bitten man.

A number of other tracings are available to show the influence of Cobra venom on the heart of cats and rabbits in strong solutions (1/5000 to 1/50,000). In every one of them the inclination of the heart to pass into firm systolic tone is clearly shown, whilst most of them display a distinct tendency to quickening of the beat very soon after the heart is affected by the venom. The early phase of excitement and the rapidly following failure are also constantly in evidence. We may summarise the action of Cobra venom on the isolated mammalian heart, thus :—

(I.) Strong solutions kill the mammalian (cat or rabbit) heart very quickly in a position of systolic tone, both chambers being similarly affected.

(II.) The death of the organ is preceded by a phase of excitement, during which the heart movements are greatly exaggerated.

(III.) During the stage of excitation the rate of beat is sensibly increased, though this is obscured by a fall in the temperature of the perfused fluid, that fall being due to obvious instrumental defects, which are difficult to eliminate. The influence of the venom on the intra-cardiac terminals of the inhibitory mechanism may, and probably does, play an important part in these experiments.

(IV.) The condition of systolic tone, and almost certainly also, the constriction

of the coronary vessels, produced by the circulating venom, greatly interfere with the circulation through the heart-tissues.

(V.) Less strong solutions of Cobra venom appear to have a stimulating and steadying effect on the heart, but even so dilute a solution as 1/500,000 gives rise to a preliminary phase of excitation, and tends in the end to kill the organ.

(VI.) The greatly increased amplitude of cardiac excursus seen in the excitation phases of these tracings, would seem to indicate, as suggested in para. III. above, that two distinct influences are at work on the mammalian heart, when it is under perfusion with solutions of Cobra venom. These are (1) the direct action of the venom on the muscle or on the nerve terminals which convey impulses leading to contraction, and (2) its action on the terminals of the vago-inhibitory mechanism. The former tends to throw the heart into strong systole, whilst the latter draws it towards diastole.

Blood-Pressure Experiments on Rabbits.

Early in 1903, Dr. PRENTICE and I made a number of blood pressure experiments on cobraised rabbits under chloroform. Each tracing was subsequently analysed by me in the following manner. At short and regular intervals, the blood pressure was estimated in millims. of Hg, the heart beats and respirations were counted for the corresponding 10 secs., and the amplitude of the respiratory excursus was measured. The amplitude of the beat was not measured on the tracings, since it is no criterion of the force of the heart. The details thus obtained were then mapped out on charts, so that a comprehensive survey of the whole experiment could be easily and quickly obtained. It would be impossible within the limits of this communication to do more than give a summary of the results obtained.

The tendency of most of the past work on the subject has been to give doses of snake venom which proved fatal in very short periods of time (ranging usually from 10 mins. to 1 hr.). We desired to avoid this as far as possible, bearing in mind that such poisons take, as a rule, from 3 hrs. upwards to kill, and that it was therefore unsafe to draw deductions from what must obviously be extreme overdoses. We accordingly endeavoured to graduate our doses so as to kill in periods of from 5 hrs. downwards. Intravenous injections were made almost invariably.

It was observed that when a relatively large dose of Cobra venom was given (one that would prove fatal in an hour or less), the blood pressure fell rapidly and suddenly, whereas when the dose was graduated to kill in the course of several hours, little or no primary fall took place. Whether there was an initial fall or not, the blood pressure subsequently rose in most cases to or beyond its previous level, which it frequently greatly exceeded during the asphyxial stage which heralded death. Rarely there was no recovery to speak of after the first fall of blood pressure; this occurred when doses were given which proved fatal in 20 mins. or

less. When the dose was a low one and gradually given, the blood pressure rose steadily from the first, and sometimes attained very high levels. A point of great interest was that it was impossible to trace any sufficient relationship between the early falls of blood pressure and changes in the rate of the heart beat at the time. For instance, in one case a rabbit received half the subcutaneous M.L.D. intravenously, and the blood pressure fell from 108 to 55 millims. in the carotid, and again recovered till it stood at 98 millims. The whole took place in 4 mins. and the rate of heart beat only varied from 41 to 39 in the time. Obviously the force of the beat must have varied greatly, or some other powerful factor must have come in.

The general tendency in these tracings was for the rate of heart beat to decrease steadily till near the end of life, when the fall became suddenly rapid, and was accompanied by a corresponding rapid descent of blood pressure. Occasionally a slight rise in cardiac rate was observed for a short time after the injection of the venom; this only occurred when low lethal doses were given.

The sudden final fall of blood pressure was the more remarkable, since in most cases the pressure was very high just before it occurred, though the heart beat had been diminishing steadily in frequency for a long time previously. The final run down of these two curves (blood pressure and heart rate), strongly suggests cardiac failure as the cause of the ultimate collapse of blood pressure.

To what was one to ascribe such failure?

The following suggested themselves as its possible causes:—

- (1) Inhibition of the heart by the direct action of Cobra venom on its vagal inhibitory mechanism.
- (2) Inhibition due to asphyxiation of the same mechanism, as an outcome of respiratory failure.
- (3) Direct or indirect action of the venom on the cardiac muscle or on its vagal ganglia.
- (4) Direct or indirect interference with the vaso-motor centre, leading to vasodilatation of the splanchnic area, and possibly
- (5) A contributory reflex inhibition of the heart as the result of high arterial pressure.

In order to throw light on these obscure points, we adopted a number of devices.

(A) We tested the activity of the vaso-motor centre in the rabbit, by stimulating in turn the depressor and sciatic nerves in animals which were in various stages of cobraism. We found that a marked response was elicited in either case up to the very end of life. There was, therefore, a strong presumption that the vaso-motor centre was not markedly affected by Cobra venom, though the TRAUBE-HERING curves which we noticed as a common feature in our tracings, suggested that it was in a state of excitement, probably as a result of asphyxiation. It is just possible that the leakage of magnesium sulphate into the blood may be sufficient to account for the presence of these curves.

(B) We cut the two vagi at various stages of our experiments, and observed the effect. As is well known, the vagal inhibitory mechanism of rabbits is not very active. We found comparatively little change produced by section of these nerves before the injection of venom or even after the injection, provided that marked slowing had not yet occurred. Later, however, the section of the vagi caused a distinct rise, both in the rate of the beat and in the height of the blood pressure. When the section of the nerves was made during the final fall of pressure, it raised the rate of beat slightly, but the pressure not at all. It is noteworthy that preliminary section of the vagi did not prevent the ultimate cardiac slowing, though it appeared to distinctly delay its evolution. Stimulation of the vagi always caused active inhibition, thus negating any idea of paralysis of the vagal nerve-end mechanism in the heart.

(C) We injected small doses of atropine sulphate intravenously at various stages of our experiments, and observed the effects. Before marked cardiac slowing had set in, the result of these injections was inappreciable, but later, when the rate of beat had fallen considerably, each injection was followed by a distinct quickening of the heart, a quickening which seemed to hold some relation to the amount of slowing which the venom had previously produced. In no case, however, was the rise in rate associated with a corresponding rise in blood pressure; on the contrary there was always a fall in pressure, following each injection.

The apparent conclusions were as follows:—

(1) The vaso-motor centres were not responsible for any of the main features of change in a blood pressure tracing of an animal poisoned by Cobra venom.

(2) Inhibitory impulses descending the vagi played a part in the slowing of the heart; the vagal mechanism remained active throughout.

(3) The cardiac slowing observed was only partly due to central vagal inhibition and a farther explanation for it must be sought.

Respiration.—The respiratory movements were recorded, either by the double stethograph or by a modification of HEAD's method.

These tracings taught but little that has not already been observed as to the changes in respiration during cobraism. The main tendency was for the rate of respiration to fall, and for the type to become at first irregular and then spasmodic. This irregularity influenced both the rate and also the amplitude of respiration. As death approached, the spasmodic gasps became more and more frequent, culminating in dyspnoeal storms which were followed by periods of apparent respiratory exhaustion. Respiration always ceased before the final fall of blood pressure, and it was not uncommon to find the blood-pressure curve rise steeply just before death. This was presumably due to excitation of the vaso-motor centre by venous blood. The same cause will explain the cardiac inhibition which we have seen brought about the final fall of blood pressure.

I have dealt with these experiments apart from the rest of my blood-pressure work,

because I desire to acknowledge the assistance I received, both from Dr. SILLAR and from Dr. PRENTICE, in carrying them out. For the conclusions drawn, I must, however, take the whole responsibility.

Blood-Pressure Experiments on Cats and Dogs.

In order to follow up the subject, I undertook a further series of experiments on cats and dogs. Very few of the latter were used. Cats are preferable to rabbits, as they are more hardy, and have tougher vessels and tissues. These experiments were, by Professor SCHAFER's kind permission, carried out in the Physiological Laboratory of the Edinburgh University.

A few tracings were first taken with the object of comparing the results obtained with those already dealt with above (on rabbits). The shortest experiment lasted 40 mins. and the longest 4 hrs. An added point of interest lay in the tracings of intestinal volume taken simultaneously with the general blood pressure, by means of a modification of SCHAFER's plethysmograph. It may at once be said that all the evidence showed that the vascular pressure in the splanchnic area was affected by the venom in exactly the same way as was the general blood pressure. It in no sense reacted independently. In fact, it is possible to trace each change of general blood pressure on the plethysmographic tracings. With cardiac inhibition or with cardiac recovery they move down or up together, whilst the constriction of their respective vessels by the direct action of the venom on their walls is indicated by a downward movement of the plethysmographic lever and an upward movement of the manometer needle.

These results clearly support the conclusion arrived at by Dr. PRENTICE and myself, that the vaso-motor centre is not profoundly affected by Cobra poison, and that the final fall of blood pressure in cobraism is not due to a paresis of this centre.

This preliminary work on cats brought out a farther point of interest, namely, that in these animals the early fall of blood pressure which immediately follows the injection of a large dose of Cobra venom is due to cardiac inhibition. This is well shown by the extract (9) from a blood pressure tracing to be found on Plate VI.

The slowing of the beat is well marked. I presume that the absence of this feature dwelt on in rabbits is to be explained by their lessened activity of cardiac inhibition. This question of inhibition will be dealt with more fully in the next section.

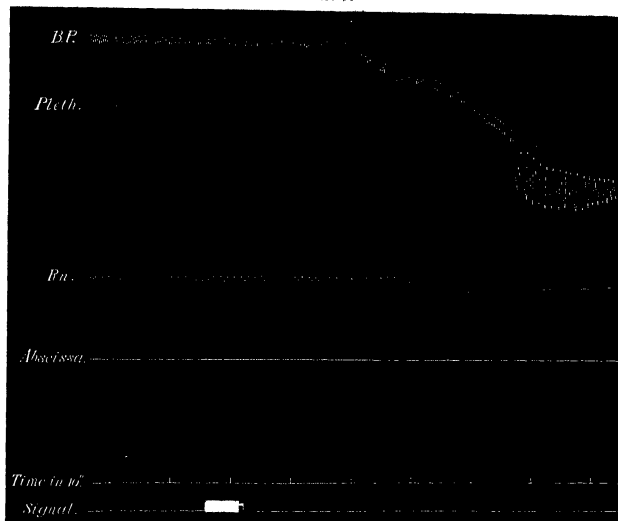
Experiments on the Exposed Mammalian Heart (in situ).

My next step was to study the effect of venom, with the chest opened. Artificial respiration was kept up from the first; a portion of the thoracic walls was removed to expose the heart, and the movements of the auricle and ventricle were conveyed to two levers by means of silk threads working over pulleys and attached by hooks to the auricular and ventricular walls. The blood pressure in a large artery (carotid or femoral) was at the same time recorded, and in a few cases the vascular changes of

volume in a loop of small gut was also taken by means of a plethysmograph. Care was taken to fix the heart steadily by means of a pair of forceps, clamped in place, and taking a good hold of the fat at the auriculo-ventricular junction. The animal was carefully kept warm throughout the operation.

In some of the experiments, large doses of venom were given in order to study the cause of the blood-pressure fall which has been already alluded to as occurring under

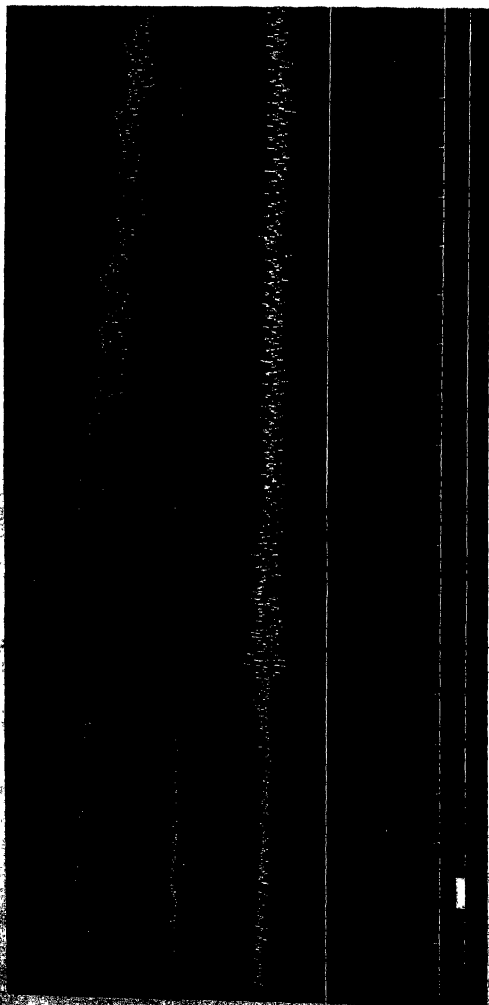
Extract 9.



Extract from Plate VI. A male cat, weight 2 kilogrammes, received intravenously one-sixth of the subcutaneous M.L.D. at the point signalled. B.P. = blood-pressure trace; Pleth. = record of intestinal volume taken in Brodie's plethysmograph; Rn = respiratory trace taken by a double stethograph. Note the evidence of cardiac inhibition and respiratory failure quickly following the injection of venom.

such circumstances. Plate 7 throws a good deal of light on this subject (Extract 10). In it extracts are given from two tracings, one of which was taken from a dog and one from a cat. The doses of venom given were speedily fatal, though the dog rallied temporarily, as shown in the plate. In both cases it will be observed that marked inhibition took place, the auricle stopping in complete diastole, and the ventricle beating very irregularly. On the same plate will be found analyses of the whole of the two experiments. (Full explanations accompany each chart, but it may be said that throughout this paper all tracings are to be read from left to right; systole is always upward; the charts analysing tracings are kept on the same plan throughout, the same colours being always used for the same purpose, blood pressure

Extract 10.



Extract from Experiment II., Plate VII. A dog, weight 10·7 kilogrammes, received intravenously one-fourth the subcutaneous M.L.D. at the point signalled. The traces in order from above are blood pressure, auricular beat, ventricular beat, abscissa, time in 10', and signal. Note the marked cardiac inhibition.

is always estimated in millims. of mercury; heart-beat rate in beats per 10 secs.; respirations in respirations per 10 secs.; the auricular and ventricular excursions are charted by multiplying the actual by 10, and stating in millims.).

To what is this cardiac inhibition* due? Clearly it is not brought about by asphyxiation of the vagal inhibitory mechanism, for the animal was under artificial respiration throughout in each case. Farther, if one refers again to Plate VI., extract 9, it is obvious that the failure of respiration cannot wholly account for the inhibition seen, though it may have contributed to produce it; for the cessation of breathing precedes the manifestation of cardiac failure by too short an interval.

Returning to Plate VII., it is observed that in extract 10 (the cat) the auricle was inhibited and the ventricle became feeble and irregular. The latter appeared to have taken on the independent rhythm characteristic of the ventricular beat, when removed from the control of the auricles. In extract 2 the auricular inhibition is early and complete, while the ventricle passes into an even more pronounced stage of irregularity than that of the cat. It is particularly noticeable that we have here to do with *distinct inhibition*, the auricle stopping in diastole, and the ventricles tending to do the same.

If now we turn back for a moment to the work on the isolated frog and mammalian hearts, several points of interest present themselves. An easy calculation shows that the concentration of venom in the two experiments of Plate VII. was below $1/30,000$; in fact, it was about $1/10,000$ in the dog, and $1/18,000$ in the cat. These calculations omit to make allowance for the fact that at first, at any rate, the venom could not have been distributed throughout the entire volume of blood. The strength of solution must therefore have been greater than the calculation shows. Such a concentration of Cobra venom would, as we have seen, kill a frog-ventricle in a few minutes, acting on its muscular tissue, and throwing it into systolic spasm. On the mammalian heart we have observed that the same tendency to pass into systolic tone is manifested, but that it is largely masked in the early stages of the experiments by the violent excitation of the heart muscle, and possibly also by the action of the venom on the terminals of the vago-inhibitory mechanism in the heart. Of this, more later on. We noticed that the isolated mammalian heart took much longer to be killed than the frog-heart, when the same strengths of venom were used, and that the marked quickening of the beat, which was so obvious a feature in the latter, was masked in the former, partly, no doubt due to the cooling of the perfused fluid, when obstruction was offered to its outflow. The influence of the action of the venom on the terminals of the vagal inhibitory mechanism must not, however, be lost sight of.

The differences between the behaviour of the isolated heart and of the heart *in corpore*, which we have just been reviewing, show that two active and opposite

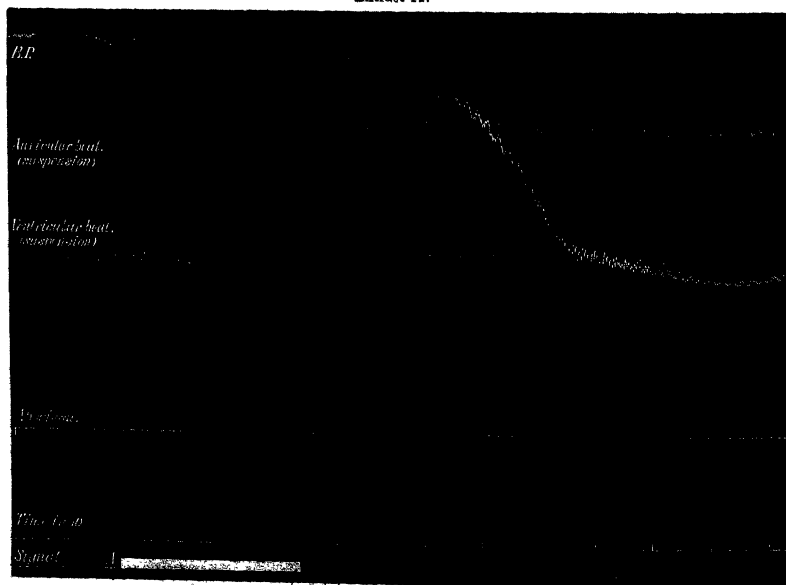
* Throughout this paper I have ventured to include two elements under the term "cardiac inhibition," viz.: (1) A decrease in heart-rate, and (2) A diminution in heart-force.

forces are called into play by the injection of Cobra venom. On the one hand we have the direct action of the venom on the heart muscle throwing it into systolic tone, and on the other we have evidence of inhibition powerful enough to mask and overcome this direct action. The rapidity of the inhibition was suggestive of central action, and in order to test this point the vagi were cut in animals whose hearts had been inhibited by the action of various doses of venom.

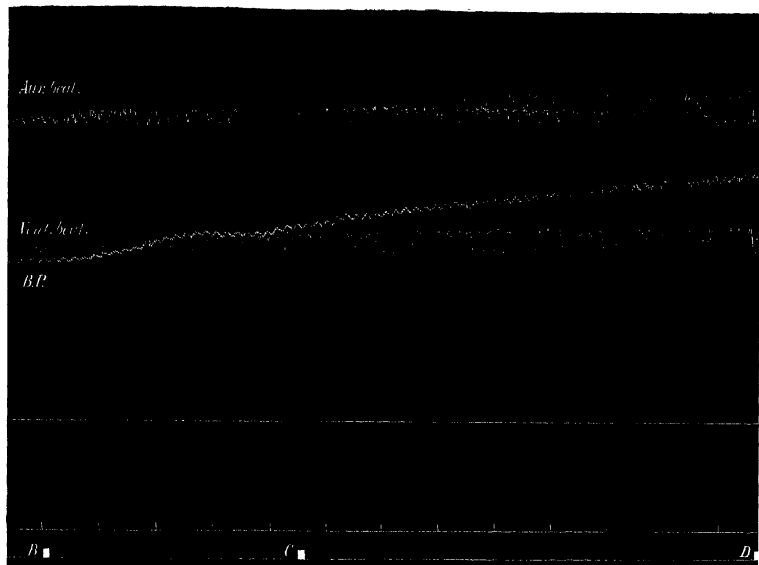
Plate VIII. contains extracts from three such experiments. Extract 1 is from a cat which had received intravenously one-seventh of the subcutaneous M.L.D.; inhibition of both auricle and ventricle was marked, but not complete; section of the nerves produced a striking effect, for not merely was the inhibition removed at once, but the beat became much stronger than before. The last circumstance I attribute to the action of the venom on the heart muscle, which has already been noticed in connection with the isolated heart.

Extract 3 is from a similar experiment in which the dose of venom was much lower, being only one-twelfth of the subcutaneous M.L.D. The fall in blood pressure was less marked and longer delayed, and inhibition was less complete; the auricles were very distinctly affected, the ventricles decidedly less so. Here, again, the influence of section of the vagi was immediate and unmistakeable; the inhibition, brought

Extract 11.



Extract 11—continued.



Extract from Experiment 1 of Plate VIII. The traces in order from above downwards are (1) blood pressure, (2) auricular beat, (3) ventricular beat, (4) abscissa, (5) time in 10", and (6) signal. (A) marks the injection of venom, (B) the section of the left vagus, and (C) that of the right vagus.

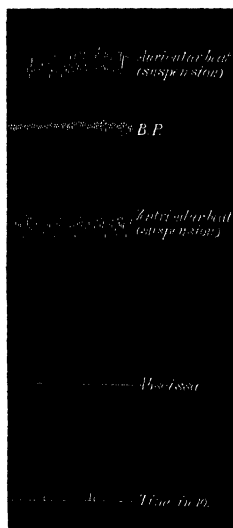
about by the injection, was at once removed for the time, at least. An analysis of the experiment is shown at length on Plate VIII. (see back thereof), and it will there be seen that inhibition reasserted itself, and was again checked late in the experiment by the division of an overlooked strand of the right vagus.

Extract 2 of Plate VIII. shows that in the cat, late section of the vagi produces a much less pronounced effect than an early section. This must not, however, be taken as in any sense contradictory of the results in rabbits, for we are not now dealing with the effect of Cobra venom on an animal which is being gradually killed by an ordinary lethal dose, but with the effects of a dose which would long before have proved lethal had not artificial respiration prolonged life far beyond its ordinary term. Here the question of nerve-end paralysis comes in.

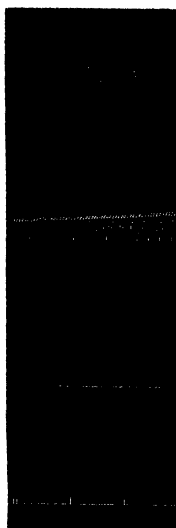
A farther light on the subject is obtained by study of the extracts from the tracing on Plate IX. The complete analysis of the tracing is also given. A dose of venom corresponding to one-eighteenth of the subcutaneous M.L.D. was first given intravenously, and subsequently (the vagi remaining intact) small doses (half the first each

time) were injected in the same way. It is abundantly clear that there are two forces pulling against each other, viz., inhibition lowering the force of the beats, and a direct muscular action increasing their force.

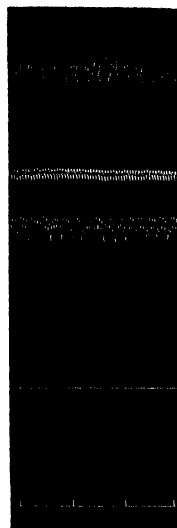
Extract 12.



Extract 13.



Extract 14.



Extracts 12, 13, and 14, from Plate IX. The traces in order from above down are (1) auricular beat (2) blood pressure, (3) ventricular beat, (4) abscissa, (5) time in 10". The first extract is taken before the administration of the second small dose of venom, the second 8' after this injection, and the third 5' later still. Note that the blood pressure first falls and then rises, whilst the auricular and ventricular beats vary correspondingly in their amplitude.

Plate XI affords an interesting contrast to the last. Here we have practically the same experiment, with this important exception, that the vagi were divided after the first dose of venom. A steady diminution of the beat-excursus is here observed, but the up and down character of the chart is lost. The only apparent explanation is that the vagal impulses of central origin, which are sent out under the influence of cobraism, have been cut off.

How, then, are we to explain the fall of blood pressure, beat-rate, and beat-force, which occur even after division of the vagi. Due allowance being made for the shock inseparable from so severe a procedure as opening the chest, I think there still remains a residue to be accounted for, and the only explanations that offer themselves are (1) exhaustion of the muscle (which is indicated as probable in the late stages of

experiments with isolated hearts), and (2) stimulation of the vagal terminal apparatus in the heart, as suggested by BRUNTON and FAYRER. If such a stimulation really takes place, it would be not unlikely that some evidence of nerve-end affection could be obtained by giving an animal large doses of Cobra venom, and keeping it alive for long periods by artificial respiration. I tried this on several occasions, and the result was always the same. The vagal nerve ends were not paralysed, but they were always greatly dulled. Plate X. illustrates this, and I think it will be admitted that after every allowance has been made for fallen blood pressure, for repeated stimulation, etc., the nerve-end mechanism shows decidedly the effect of the venom.

Experiments to Test the Action of Artificial Respiration with the Chest-Walls Intact.

I next turned my attention to the influence exerted on the heart, and on blood pressure, by artificial respiration used with the chest walls intact, after ordinary respiration had failed. The experiments were of great interest, and I, therefore, give them each in turn, but as shortly as possible.

Plate XII.

Dog, weight 5·7 kilos.; chloroform by tracheal tube; blood pressure taken in femoral; respiration recorded by a stethograph clamped in position on the chest wall; intestinal volume by the plethysmograph; all injections of venom solution made by a cannula tied into the external saphenous vein (the venom was dissolved in RINGER's fluid, as usual in my experiments, '0005 grammes = 1 c.c.).

In all, a little over one-fourth of the subcutaneous M.L.D. of venom was injected in rather more than 70 mins., the injections being given at regular intervals. Eighty-four mins. after the first injection respiration had failed, having ceased 2 mins. earlier, the heart-beat had fallen from 21 to 22 in the 10 secs., and blood pressure was at danger point. Artificial respiration was at once commenced, and the recovery of heart-rate and blood pressure was immediate; the former went up to 28 per 10 secs., and the blood pressure shot up higher than it had yet been during the experiment. After 13 mins. the artificial respiration was stopped, and in less than 12 mins. the blood pressure was at zero, the heart had stopped, and the animal was dead. A complete analysis of this experiment is given on Plate XII., and extracts from the most interesting portions of the chart are also furnished there. A point to which I draw special attention is the partial recovery of respiratory function after a period of artificial respiration.

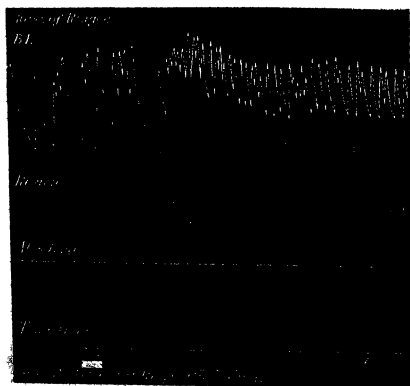
Plate XIII.

The next experiment closely resembled the last. The dog weighed 5·5 kilos.; no plethysmographic tracing was taken; the first injection was a very large one,

corresponding to five-sixths of a subcutaneous M.L.D. of Cobra venom. This was given by the external saphenous vein, as in the last case. The rate of heart-beat ran quickly up, and then as quickly went down again; blood pressure fell steeply; there was evident strong inhibition; respiration showed a short, sharp check, which will not, however, account for the inhibition, for it is too late, and too transient after a temporary recovery; a late fall of respiratory force and frequency set in, ending in complete failure, which necessitated the use of artificial respiration 36 mins. after the injection had been made. The heart-beat fell rapidly toward the end, reaching 3 in 10 secs., and there was an asphyxial rise of blood pressure. Artificial respiration steadied the blood pressure, and the heart-beat at once rose to more than its former level. A second injection of venom (half the previous one in strength) brought the heart-rate down from 23 to 24, and the blood pressure also began to fall. Section of the vagi quickly sent the beat up again to about its previous figure. From this time on, an interesting feature appeared on the tracing, for the beats occurred in phases which alternated with each other. In one phase the beats were exactly double those in the other. Evidently 1 beat out of 2 tended to be suppressed, or, rather, failed to be able to pass some obstructive barrier, probably at the auriculo-ventricular junction.

Three farther doses, each of the same strength as the last, were now given at intervals, the last two being injected, with only 2 mins. between them; yet there was no further evidence of inhibition beyond an increasing tendency for the phases of slow beat to lengthen to the exclusion of those of the faster beat. Blood pressure

Extract 15.

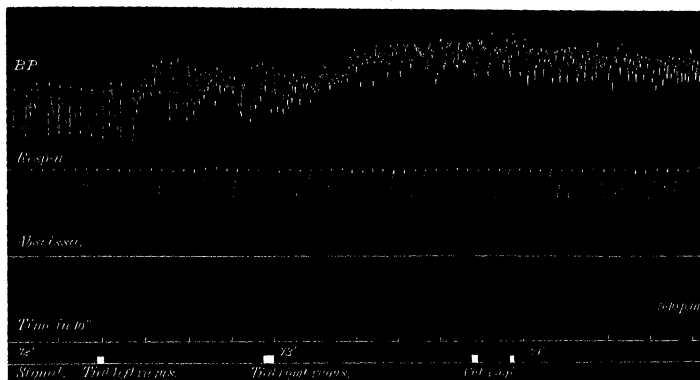


Extract 16.



Extracts 15, 16, 17, and 18 from Plate XIII. The traces in order are (1) blood pressure, (2) respiratory movement, (3) base line, (4) time in 10". No. 15 is between the 35th and 38th minutes, No. 16 at the 45th minute, No. 17 between the 72nd and 75th minutes, and No. 18 between the 96th and 98th minute. A reference to the text explains these extracts. (See also Extracts 17 and 18.)

Extract 17.



Extract 18.

Respiration

Pulse

Time to 10''

Normal - 23''

remained high. To end the experiment, a dose double the first one was injected, and the animal was dead in a few minutes, with its heart tightly contracted, and very hard.

Plate XIII. gives all details of this experiment, which appears to prove (1) that the primary fall of blood pressure, which follows the injection of a large lethal dose of venom, is not due to respiratory failure alone; (2) that Cobra venom in large doses can, and does, act centrally on the heart through the vagal nerves; in other words, it has a direct action on the cardio-inhibitory centre; and (3), that, provided efficient artificial respiration is kept up with an intact chest, an animal is capable of resisting

for some time the action of enormous doses of the poison. At the same time, the frequent dropped beats seen on this tracing show that some measure of cardiac inhibition was present; the curious phases alluded to confirm one in this belief. Advantage was taken of this experiment to test the activity of the vagal nerve-ends from time to time. Extracts illustrating this point will be found on the back of Plate XIII, and the matter will be again returned to later on. For the present, it suffices to say that nerve-end paralysis was evidently beginning here.

The next experiment proved an instructive failure. A cat (weight 3 kilos.) received five-eighths of the subcutaneous M.L.D. intravenously in 65 mins., through the external jugular vein. The dose was given in ten slow injections. As seems to be the usual case, when the venom is thus administered, there was a steady fall of blood pressure following the first rise. Respiration became convulsive, and artificial assistance to breathing was delayed too long, and was, thus, of no avail. After several such failures, one learnt when to interpose with a good prospect of success, and yet without exercising undue haste.

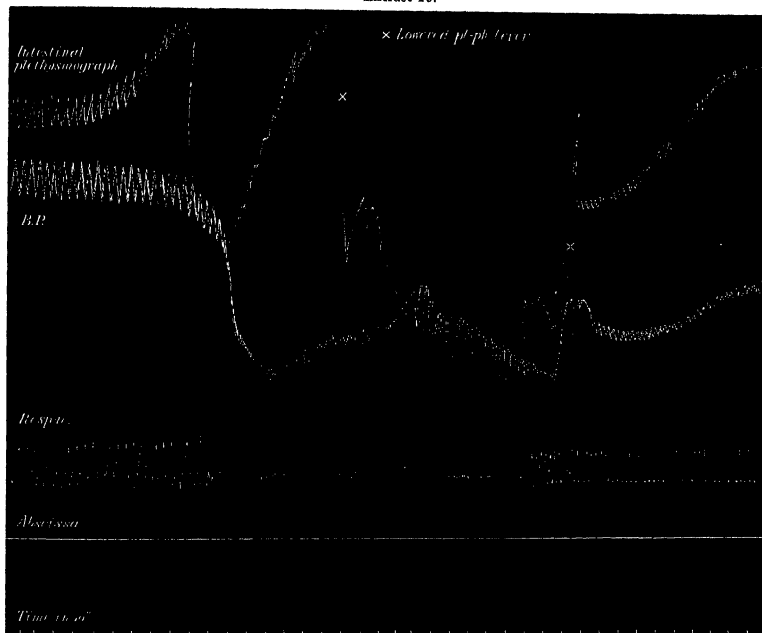
Plate XIV.

A cat (weight 3·4 kilos.), received three-fifths of the subcutaneous M.L.D. of Cobra venom intravenously, in 21 injections given at 3 mins. intervals. The blood pressure fell steadily, and artificial respiration had to be begun 69 mins. after the first injection. A control animal weighing 2·3 kilos. received the same proportionate dose similarly spread over an hour, and was dead 69 mins. after the first injection. As in the last experiment cardiac inhibition was evidently the cause of (threatened) death. Respiratory failure was in evidence, but had not become complete. The animal was unconscious. Artificial respiration raised the rate of heart-beat, partially restored the fallen blood pressure, and brought the cat back to consciousness. Moreover, instead of dying in about 70 mins., the animal lived on nearly 4 hrs. longer. Before artificial respiration was begun, occasional dropped beats were observed, later these became still more frequent, and the irregularity of the blood-pressure curve showed that the heart was acting very unevenly. At the same time respiration was failing in spite of the artificial aid it received. Towards the end of the experiment, there was almost no respiratory effort, and cardiac inhibition was very pronounced. For analysis of the tracing and for extracts therefrom, see the original Plate XIV. I will revert to discuss this, together with the next experiment, later on.

A cat (weight 3·38 kilos.), received one-fourteenth of the subcutaneous M.L.D. of Cobra venom intravenously in one dose. There was a very rapid fall of blood pressure, with marked cardiac inhibition. Respiration failed whilst the blood pressure

was falling, and it appeared as if this failure was final. Artificial respiration was at once commenced (with the pump attached to the motor apparatus). The blood pressure and heart-beat at once began to recover, and reached nearly average levels. From time to time, artificial respiration was suspended. Each time this was done, there was a steep rise of blood pressure, and a fall in the number of beats; these were plainly asphyxial phenomena, occurring through the vaso-motor and vagal centres, and were speedily removed by a return to artificial respiration. During the earlier periods in which artificial aid was withdrawn, little or no respiratory effort was noticeable, but in time the oxygenation of the blood appeared to have restored the

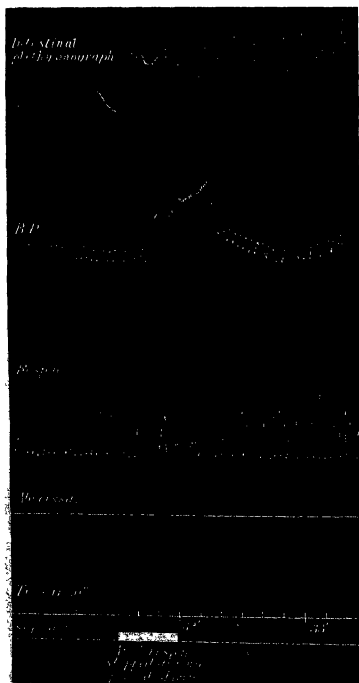
Extract 19.



Extracts 19, 20, 21 and 22 are from Plate XV. The traces in order from above down are (1) intestinal volume by plethysmograph, (2) blood pressure, (3) respiratory movements, (4) abscissa, (5) time in 10", (6) signal. In Extracts 20, 21, and 22 the continuous signal shows the suspension of artificial respiration. The time in minutes after the injection is shown on the time trace. Extract 22 shows the last 5 minutes of the experiment. A reference to the text explains the extracts. (See also Extracts 20, 21, and 22.)

respiratory mechanism to activity. This restoration was a gradual one and may plainly be traced on Plate XV., both in the analysis and in the extracts from the tracing. The suddenness of the primary cessation of respiration, and the short period which elapsed before the commencement of the recovery of respiration, point unmistakably to the action having been a central one, for there would not have been time for nerve-end paralysis to have occurred. To this point I shall recur later. The experiment was brought to an end 3 hrs. after its commencement, by division of the

Extract 20.



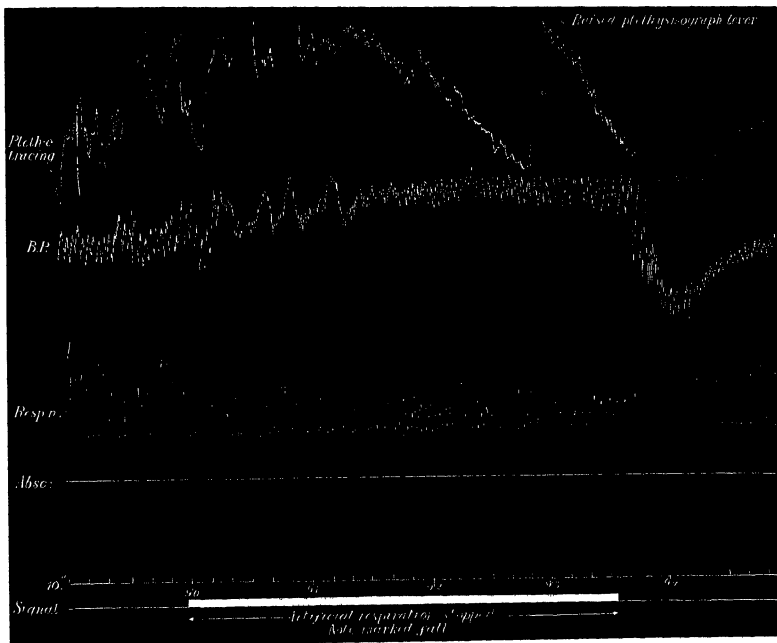
carotid, in order to allow the laboratory to be closed. During the last half hour of that period the animal was breathing freely and spontaneously, unaided; blood pressure and heart rate were very steady, and the animal required constant anæsthetising to keep it unconscious. Whether or not nerve-end paralysis would have eventually closed life it is impossible to say, but it is clear that the artificial aid rescued the respiratory centre from death, and enabled the animal to be alive and functionally active nearly 3 hours after it would otherwise have been dead.

The Effect of Cobra Venom on the Intra-Cardiac Vagal Nerve-Ends.

The present seems to be a suitable opportunity to pause to examine the evidence in favour of a paresis of the vagal nerve-ends in cobraism.

(1) Dr. PRENTICE and I found, in one blood-pressure experiment on rabbits, that the cutting off of central vagal impulses from the heart, either by the severance of the vagi or by the use of atropine solutions, would check, but not stop, inhibition. The rate of beat declined, and the blood pressure fell in spite of these measures.

Extract 21.



(2) Again, in experiments on cats and dogs with the front of the chest removed and with artificial respiration maintained throughout, I observed that though section of the vagi played a much more pronounced part here than in the rabbit experiments, it was not sufficient to stop the onward progress of inhibition, though it undoubtedly greatly retarded it.

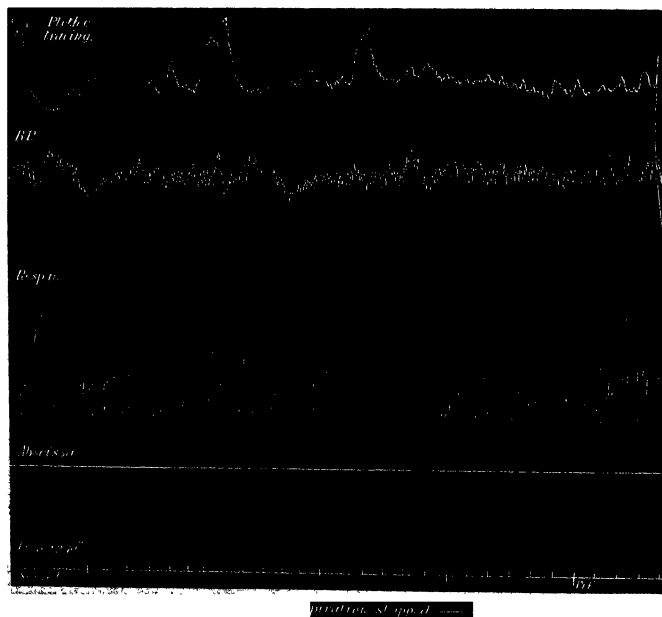
(3) The experiments with artificial respiration, used with intact chest-walls, threw a farther light on the subject. They showed that the free oxygenation of the blood

could retard the action of the venom on the centres, or at least could maintain those centres in activity, but they suggested that it had less power to stop the paralysis of the vagal and phrenic nerve-ends by the poison. Given sufficient time, the blunting of the vagal nerve-ends was seen to occur, whether the chest was opened or not.

Here, again, inhibition progressed though slowly after section of the vagi.

(4) The difference between the behaviour of the isolated hearts of the frog and mammal is suggestive. In the case of the former, we have the ventricle alone exposed to the venom, whilst in the latter the poisonous fluid traverses every portion

Extract 22.



of the organ, and acts unrestrainedly on the terminals of the cardio-inhibitory mechanism. In any case, we find in the cat and rabbit heart under perfusion a greater resistance to the venom, and a much lessened, though ever present, tendency for the organ to pass into systolic tone.

The whole evidence, therefore, points to the venom acting on the terminals of the cardio-inhibitory mechanism directly, and it is also probable that it has a farther indirect action on them by increasing the viscosity of the blood, which it does by its interference with the respiratory mechanism.

The Determining Factors in Respiratory Death from Cobra Venom.

BRUNTON and FAYRE came to the conclusion that Cobra venom attacked the centres in the medulla oblongata, and especially the respiratory centre. RAGOTZI urged that they had brought no experimental proof to support their contention, and contrived an ingenious but complicated experiment to show that it was not the respiratory centre, but the phrenic and other motor nerve-ends which were at fault. He found that two factors were necessary to bring about such a nerve-end paralysis, viz., a sufficiently large dose of venom, and a sufficiently long period for that venom to act. If too large a dose of venom were given, he found that death occurred before the motor nerve-ends were paralysed, whilst if the dose were too small, it failed to produce the effect. His crucial test was made as follows :—A rabbit was fixed on its back, with its neck grasped by a fork-shaped iron, whilst an iron ring embraced its muzzle, pressing on the nasal bones and jaw in such a way, according to RAGOTZI, that the access of the poison to the nasal muscles was delayed, with consequent delay of their paralysis. The rabbit was then poisoned intravenously, and when complete motor paralysis had occurred, artificial respiration was commenced. So long as the animal was apnoëic, no movement of the nostrils was observed, but when artificial respiration was suspended, the nostrils began to move, at first gently and later in a more exaggerated fashion; convulsions of the posterior extremities also occurred. Under artificial respiration these nasal and other convulsions subsided. This experiment led RAGOTZI to conclude that the respiratory centre remained active after the motor nerve-ends of the muscles of respiration had been paralysed.

I have shown in the preceding pages that the injection of a large dose of Cobra venom intravenously causes an abrupt stoppage of respiration, side by side with an equally abrupt inhibition of the heart. In the experiment shown on Plate VI., the cessation of respiration lasted nearly 3 mins., then a number of inspiratory gasps occurred, heralding a rise in blood pressure, and after this respiration gradually re-established itself, and the animal lived over an hour in spite of a farther dose of the venom. Here there was clearly no time for the nerve-ends to have become affected, and the action was obviously a central one, for RAGOTZI found that rapidly lethal doses do *not* paralyse the phrenic nerve-ends, and it will be seen directly that my own work bears him out in this.

In the experiment given on p. 392 and on Plate XV., it will be remembered that there again respiration was abruptly stopped by a large intravenous dose of Cobra venom, and that apparently the animal would have speedily died but for the use of artificial respiration. Here we have a condition of affairs resembling that in RAGOTZI's experiment. The paralysed centre was restored to activity by artificial aid; consequently, the rises in blood pressure and the struggles of dyspnoea which RAGOTZI observed are likewise seen here. Moreover, in my experiment the evidence

of central activity became more and more obvious as the beneficial effects of artificial respiration had time to assert themselves.

RAGOTZI asserted that in his experiment the motor nerve-ends were paralysed, though he obviously can have had no absolute proof that this paralysis extended to the phrenic terminals. He called for experimental proof that the respiratory centre was affected by venom. To comply with this reasonable demand, I undertook a number of experiments, which I will arrange in series, giving a short outline of each such series.

Series No. 1.—To test the action of Cobra venom on the respiratory centre when it is directly applied to that centre.

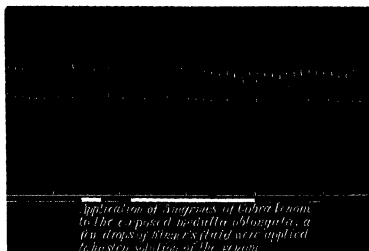
Several experiments were performed, and always with the same result. In each case a rabbit, weighing a little over 2 kilos., was chosen for the experiment, and the venom used had a subcutaneous M.L.D. for the rabbit of '0006 gramme per kilo. The animal was etherised, and the anæsthetic was administered by a tracheal cannula, in order to secure evenness of administration. The medulla oblongata was exposed in the usual way, by removal of the posterior occipito-atlantoid ligament, after cutting through the superjacent structures. Respirations were recorded by means of a double stethograph, and in some of the experiments the blood pressure was also taken. Five milligrammes of dry Cobra venom were dropped on to the medulla oblongata, over the seat of the respiratory centre, and a few drops of RINGER'S fluid were added to hasten solution. The result in each case was speedy death by respiratory failure. A *post-mortem* examination was held at once, and the heart was always found to be beating actively and freely; there was no tendency for it to be killed in tight systole, as would have been the case had the large dose of venom produced death by its absorption into the system. The phrenic nerves responded readily to very weak stimuli, but no contraction of the diaphragm was produced by stimulating the medulla oblongata with even the strongest currents available.

Plate XVI. contains a tracing from one of these experiments, in which the blood pressure has been purposely omitted to save complication. It will be observed that death occurred 12 mins. after venom was applied. One must remember that Cobra venom when dried does not very quickly pass into solution, and looking at the curve it is probable that about half that time (6 mins.) was really occupied by the action of the venom.

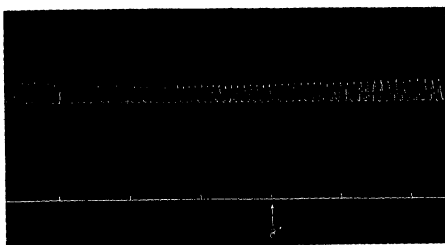
Series No. 2.—To ascertain whether diaphragmatic respiration ceased before the other respiratory movements. Two rabbits were used; each was etherised, and a tracheal cannula inserted. The chest movements were registered by means of a double stethograph and tambour, whilst the diaphragmatic movements were registered by a modification of HEAD'S method.

The first rabbit weighed 2.14 kilos., and received five-sixths of the subcutaneous M.L.D. intravenously in 34 mins. Respiration failed rapidly, and the animal was dead 56 mins. after the commencement of the injection. The diaphragm appeared

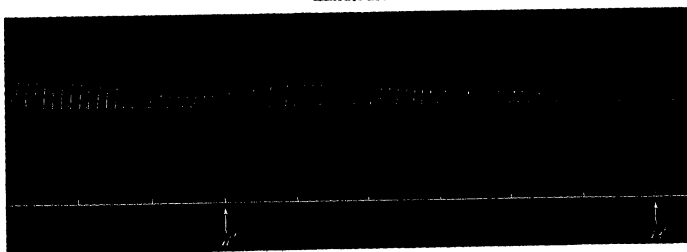
Extract 23.



Extract 24.



Extract 25.



Extracts 23, 24, and 25 from Plate XVI. Extracts from a stethographic tracing in a rabbit, whose medulla oblongata was exposed and Cobra venom applied thereto. No. 23 is at the time of application of venom, No. 24 is 8 minutes later, and No. 25 is just before and at death. The time in minutes is shown below.

to be active till the end, and the phrenic nerves retained full sensitivity; they were tested by make-and-break shocks; twitches of the diaphragm could be readily got with the secondary coil 330 millims. from the primary (the tongue could appreciate the current at 180 millims., but not beyond).

The second rabbit received half the subcutaneous M.L.D. in a little under $\frac{1}{2}$ hr. and lived 2 hrs. after the first injection. The type of respiration seemed to change during the latter part of the experiment, and the action of the diaphragm appeared to have diminished. It was difficult to be certain of this, but, in any case, some diaphragmatic movement continued to the end, and the phrenic nerve-ends proved to have retained full activity, like those in the previous experiment.

Series No. 3.—To test the effect of large doses of venom on the phrenic nerve-ends.

Two cats were given rapidly fatal doses of Cobra venom intravenously, one being killed in 15 mins. and the other in 37 mins. In both the phrenic nerve-ends retained full activity. The hearts were found in tight systolic position.

A cat (weight 1·640 kilos.) received one-fifth of the subcutaneous M.L.D. of Cobra venom intravenously in 15 mins.; 13 mins. later artificial respiration was required to avert death. After 1 hr. 4 mins. of artificial respiration, the animal was killed and its phrenic nerves were tested. Make-and-break shocks were used, and a diaphragmatic twitch was obtained with the secondary coil at 70 millims., but not with a weaker current, and stimulations of the vagi stopped the heart at 140 millims. The apparatus used for these stimulations was the same throughout all these series, and could not have differed appreciably. Testing by the tongue showed that it did not change.

Series No. 4.—To test the effect of smaller doses of Cobra venom on the phrenic nerve-ends.

A cat weighing 1·720 kilos. received one and a-half M.L.D.'s of Cobra venom subcutaneously and died 4 hrs. 4 mins. later. Convulsions and cyanosis preceded death, and the heart was beating freely when the body was opened. It was, therefore, clear that death was due to respiratory failure. The phrenic nerves were tested with the secondary current, by make-and-break shocks, and contractions of the diaphragm were readily elicited up to 450 millims. distance of the secondary from the primary coil. The coil was, in fact, pulled out 100 millims. beyond its usual working distance. When the electrodes were directly applied to the diaphragm the muscle responded to make-and-break shocks up to a distance of 145 millims. of the secondary coil.

The heart was beating fairly well when it was stopped by a vagal nerve stimulus at 140 millims.

A rabbit, weighing 1·710 kilos., received one and one-third M.L.D.'s of Cobra venom subcutaneously, and was dead 4 hrs. 55 mins. later. Before death the heart slowed markedly, the animal became cyanosed, and there were pronounced convulsions. Obviously respiratory failure accounted for death. A *post-mortem* was made directly death occurred. The heart was still beating and was inhibited by a left vagus stimulation at 100 millims., having been slowed by a stimulus at 130 millims. The diaphragm responded readily to phrenic stimulation at 400 millims.

These experiments would seem to indicate clearly: (1) that the mammalian respiratory centre can be directly poisoned by Cobra venom; (2) that the phrenic

nerve-ends remain quite unaffected, or at least non-paralysed (*a*) in animals which have been poisoned by large intravenous doses of venom, and (*b*) in those which have received doses of venom subcutaneously not greatly above the lethal, and which have taken 4 or 5 hrs. to die; (3) that the phrenic nerve-ends can be paralysed by the administration of large lethal doses of venom, provided that the animal be kept alive long enough by artificial respiration.

RAGOTZI observed that the diaphragm contracted more feebly as death approached, and argued from this that the phrenic nerve-ends were paralysed. But a central paralysis might equally explain this phenomenon if it be a fact. Then again, could RAGOTZI really cut off the blood supply of the snout for a long period (such as is demanded to produce nerve-end paresis, according to his own admission) without at the same time damaging the nutrition of the nerves and muscles he was studying? He used an iron ring for the compression of the parts. In proportion as his compression was efficient, so must his interference with the healthy action of the parts have increased; if his constriction was only partial the envenomated blood was still circulating, and was obviously acting at an advantage on tissues damaged by deficient nutrition. If the constriction was complete, or if on the other hand it was very incomplete, our conclusions are still more obvious.

I believe that the true interpretation of RAGOTZI's experiment is the one I have already given, namely, that by artificial respiration the partially paralysed respiratory centre was temporarily restored to activity, just as occurred in my experiment set forth on Plate XV.

In a recent paper in the 'Lancet' (2.1.04) LAMB and HUNTER have approached this subject purely from the histological point of view, and have found definite evidence that some of the centres in the medulla oblongata, and markedly the tenth (motor) and twelfth nuclei, were attacked by Cobra venom. I may add that my own work was finished and that my conclusions had been formulated before I saw their paper. Working on entirely different lines from LAMB and HUNTER, and without any communication with them, I have been simultaneously led to conclusions which strongly support those which they have arrived at.

Whilst insisting strongly that Cobra venom has a powerful and important central action, I have also admitted the motor nerve-end paralysis on which RAGOTZI and others have laid so much stress, and have indicated the conditions under which alone I found such paralyses to occur. The very fact that they progress whilst artificial respiration is actively maintaining life in the important medullary centres, is most significant in its bearing on treatment. In the experiment shown on Plate XIV. one can trace the partial recovery of an animal from the effects of a large dose of Cobra venom; but running through the whole tracing is evidence which can hardly be interpreted otherwise than as an indication of gradually increasing interference with the passage of respiratory impulses from the centre to the muscles.

RAGOTZI has shown that, in the case of Cobra poisoning of an isolated limb, it is

possible to obtain recovery from motor nerve-end paralysis, though the process was a very slow one. The recovery took 6 days. My own work on the subject is still very incomplete, but I have been led to think that the use of artificial respiration has been too much neglected in cases of cobraism. I am aware that a commission has pronounced on the subject, and that many have dealt with it, in past years, but I am not satisfied that any series of accurate experiments has carefully determined the limitations of a method which is at once simple and always available.

This much I can now say, if artificial respiration is to save life, it must be efficient and must not be too long delayed. As a therapeutic measure in the treatment of cases of Cobra bite, it ranks only second to the use of anti-venin. Needless to say local treatment of the bite should always be employed along with it.

Summary of Conclusions.

(1) Cobra venom acts directly on the muscular tissue of the blood vessels, or through their vaso-motor nerve-endings, constricting the arterioles, and thus raising the arterial blood pressure. It probably affects all organs alike. In the frog-vessels the action can be traced down to dilutions of 1/10,000,000. In a Cobra-bitten man, the concentration of venom in the blood is probably, at least, thirty times as great as this.

(2) Cobra venom also acts directly on the isolated frog-ventricle, killing it in a position of firm systole, if the solution be concentrated, and stimulating it if a weaker strength be employed. The limit of the speedy lethal action on the isolated heart is reached at a concentration of about 1/500,000. The stimulating action can be traced down to a dilution of 1/10,000,000. This action of Cobra venom brings it into line with the glucosides of the strophanthin group. Its action is more rapid than that of strophanthin, and is certainly not inferior to it in strength. Atropine sulphate and Cobra venom, when acting in the same solution, intensify each other's action, and produce more summation of effect than one would have anticipated. This detracts from the value of the atropine salt in the treatment of cobraism and makes it a dangerous remedy. The blood-pressure work has confirmed this view of the case.

(3) Cobra venom powerfully affects the isolated mammalian heart, when solutions of it are perfused through the coronary circulation. The action appears to be a dual one, viz., (1) a direct action on the muscular fibre or on the nerve-endings, closely resembling that which is produced on the isolated frog-ventricle; and (2) an action on the intra-cardiac vagal mechanism, which makes for inhibition. The result is that, in strong solutions, we find an irregular and extreme excitation of the heart, followed by early death in a position of systolic tone. If the concentration be less, the early stage of excitement yields to a prolonged phase, in which the tonic action of the poison on the heart is most pronounced; the beat is regular, steady, and

strong. Cobra venom interferes with the circulation through the heart in a marked manner; this is probably due (1) to a constriction of the coronary vessels, brought about by the direct action of the venom on the vessel walls; and (2) to the condition of tonus into which the heart is tending to pass.

(4) When given subcutaneously in low lethal doses, Cobra venom kills by paralysing the respiratory centre. Such a paralysis is, under these circumstances, gradually evolved, and in the early stages of the process there is often evidence of a phase of stimulation, preceding the paralytic phase.

There is a gradually increasing venosity of the blood, and in consequence thereof, all the harmful results of slow asphyxiation are produced.

If life is prolonged beyond the usual term by artificial respiration, and possibly also if the dose of venom is a very low lethal one which takes many hours to kill, the phrenic and other motor nerve-ends may become paralysed, but this is certainly not an essential feature of death from lethal doses of Cobra venom, which kill within 5 hrs. I hope to make a farther communication on this subject later.

The convulsions which precede death are purely asphyxial, and can be at once stopped by artificial aëration of the blood. Each such convulsion is followed by a phase of exhaustion of the respiratory mechanism, which is almost certainly central.

If the dose of Cobra venom administered be a large one, and especially if it be given intravenously, the respiratory centre is quickly and severely affected, and respiration may cease almost at once. This cessation of breathing may be permanent, if artificial respiration be not quickly started, but if the dose be a less severe one, the rhythmic activity of the centre reasserts itself. At first there may be a number of deep spasmodic gasps, and then the movements of respiration re-begin, very gently at the commencement and gaining force as time goes on, till a normal rhythm is re-established, or even a stage of stimulation is manifested. Soon, however, the centre fails again, and all the phenomena of asphyxiation appear.

By applying Cobra venom directly to the exposed medulla oblongata of the rabbit, I have shown that the respiratory centre can be paralysed without the phrenic nerve-ends or the heart being appreciably affected.

If very large doses of venom are injected, death may take place by cardiac failure, before the respiratory mechanism has given way. We have here to do with the direct action of the venom on the heart muscle; the beats become rapid and shortened, and the heart passes into a systolic phase, in which it dies tightly contracted.

(5) Cobra venom, when given in low lethal doses subcutaneously, raises the general blood pressure. There may be a slight preliminary fall before the rise, but often this is wanting. In the absence of farther interference the blood pressure remains high till very near the end of life. In the asphyxial convulsions which herald death, a farther steep rise of blood pressure takes place; this is soon followed by a sudden and very rapid fall to death.

The high level of blood pressure is due to : (1) The direct action of the circulating venom on the muscular tissue of the arterioles, causing a constriction of these vessels, and thus opposing a barrier to the onward flow of the blood ; (2) the increased force of the heart-beat as the outcome of the direct stimulating action of the venom on its muscular tissue, and (3) the stimulation of the vaso-motor centre, as a result of the steadily increasing venosity of the blood.

The slight preliminary fall of blood pressure, which is sometimes seen, is due to cardiac inhibition, but this subject will be reserved for discussion when dealing in the next section with the action of large doses of the poison.

The late fall in the rate of the heart-beat is due to cardiac inhibition ; the latter is due to several factors :

(1) A gradually progressive asphyxiation is taking place throughout the experiment ; this affects the vagal centre in common with the rest of the nervous system ; the result is a stimulation of the inhibitory mechanism, and a consequent slowing and weakening of the heart.

(2) The direct stimulating action of the venom on the vagal inhibitory centre acts in the same direction as the asphyxiation of the centre.

(3) There is distinct evidence that even when the influence of the vagal centres is removed, inhibition of the heart continues to advance, though in a lessened degree. The obvious inference is that the vagal nerve-ends are stimulated by the circulating venom, and probably also as a result of deficient aëration of the blood.

(4) It is not improbable that a stage of exhaustion of the heart muscle follows the early stimulative action of the venom, and

(5) Exhaustion of the heart is probably predisposed to by the strain put upon the organ, in having to work for a long period against an abnormally high blood pressure.

We are now in a position to explain the sudden rapid fall of the curves of heart-beat-rate and of blood pressure, which usher in death at the close of one of these long experiments. An over-strained and weakened heart is suddenly and violently called upon to bear a farther burden, for respiration has ceased and the medullary centres are acutely asphyxiated. As a consequence there is a violent excitation of the cardio-inhibitory and vaso-motor mechanisms. The heart is slowed and at the same time has to work against a suddenly increased pressure, and it gives way. In fact, we have the phenomena of asphyxiation in their entirety.

The vessels of the splanchnic area are affected *pari passu* with those of the body generally, and they in no wise act independently. The vaso-motor mechanism remains active throughout, and is as we have seen profoundly affected by changes in the venosity of the blood.

(6) Cobra venom when injected in large doses, and especially when given intravenously, causes (1) a sudden fall of blood pressure ; (2) a subsequent rise, provided the dose has not been too large ; and (3) a final fall to zero.

The early fall is undoubtedly due to inhibition of the heart. It has been clearly

shown that this is mainly brought about by the direct action of the poison on the vagal centres in the medulla oblongata, as it occurs before the accompanying failure of respiration has had time to act. Moreover, it is seen whilst artificial respiration is being actively carried on, and can be checked under these circumstances by division of the vagi. On the other hand there can be no doubt that asphyxiation of the vago-inhibitory centre intensifies and maintains the inhibition which direct influence of the venom on the vagal centre produces.

The spontaneous recovery of respiration or the application of artificial respiration, has a powerful influence in mitigating the action of the venom on the vagal centre. In the same way artificial respiration, and to a less extent, the spontaneous recovery of respiration, appear to act beneficially on the poisoned respiratory centre.

Even if the heart is cut adrift from all central vagal impulses, whether direct or indirect, by the division of the vagi, there yet remains evidence of a continued inhibition which must be attributed to the direct action of Cobra venom on the terminals of the vago-inhibitory mechanism. This action would appear to be a direct one, but there is every probability that it is indirect as well, in other words, that it acts through asphyxiation of the vagal terminals, as well as by the poisoning of these parts by the circulating poison.

(2) When the secondary rise of blood pressure, which follows the primary fall, occurs, it is due to the same factors which determine its occurrence when small doses have been injected. It remains to explain why it is sometimes absent, brief or ill marked. The explanation is simple; it is merely a question of cardiac failure. We have seen that the direct inhibitory action of the venom through the vagal centre is capable of overcoming the tendency which the blood circulating through the heart muscle has to throw that muscle into death in systolic tone. Were it not for these two rival forces, to some extent balancing each other, Cobra poison would kill by its direct action on the heart muscle. When the doses are comparatively small or when the vagi are cut or thrown out of gear by atropine, we find the tonic cardio-muscular influence of the venom in evidence, but when the dose of venom is a large one, and especially when it is intravenously given (the vagi remaining intact), the inhibitory action overpowers the muscular excitation and failure of the heart occurs. If the inhibition is sufficiently well marked no amount of arteriolar spasm that occurs will compensate it, consequently the blood pressure falls.

When the dose of venom is a very heavy one, the direct muscular stimulation may be so intense as to overcome the maximum inhibitory impulse, and then the heart dies in systole with a quickened beat, and is found after death as hard as a contracted *post-partum* uterus. Under such circumstances, any increase in the force of the heart is temporary, for the beat is probably a very partial one; the heart passes through a stage of excitement into one of increasing systolic tonus, in which the beats are very limited in extent.

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